



Virginia Commonwealth University
VCU Scholars Compass

Theses and Dissertations

Graduate School

2011

MICROBIAL COMMUNITY FUNCTION IN FRESHWATER WETLAND SOILS: USING EXTRACELLULAR ENZYME ANALYSIS TO STUDY THE EFFECT OF MOISTURE AND VEGETATION

Aaron Porter

Virginia Commonwealth University

Follow this and additional works at: <http://scholarscompass.vcu.edu/etd>

 Part of the [Environmental Sciences Commons](#)

© The Author

Downloaded from

<http://scholarscompass.vcu.edu/etd/2526>

This Thesis is brought to you for free and open access by the Graduate School at VCU Scholars Compass. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of VCU Scholars Compass. For more information, please contact libcompass@vcu.edu.

©Aaron James Porter, 2011

All Rights Reserved

MICROBIAL COMMUNITY FUNCTION IN FRESHWATER WETLAND SOILS: USING
EXTRACELLULAR ENZYME ANALYSIS TO STUDY THE EFFECT OF MOISTURE AND
VEGETATION

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
in Environmental Studies at Virginia Commonwealth University

by

Aaron James Porter
Bachelor of Arts
B.A., Virginia Tech University, 2005

Master of Science
M.S., Virginia Commonwealth University, 2011

Major Director:
Rima B. Franklin, PH.D.
Assistant Professor, Department of Biology

Virginia Commonwealth University
Richmond, Virginia
August, 2011

Acknowledgements

This research would not have been possible without the guidance and support of Dr. Rima Franklin. I am extremely grateful for Dr. Franklin's willingness to take on a student from outside her department, for her patience and daily encouraging words of support, and her funding which allowed me to pursue research in wetland ecology, for which I have a deep passion. I would also like to thank my committee members, Dr. Roy Sabo, Dr. Edward Crawford, and Dr. Leigh McCallister, for their insight and direction in this project. Perhaps most of all, I'd like to thank Christine Prasse, who completed the first year of this study, and whose guidance was indispensable in the completion of this research. Additionally, I am grateful for the lab and field assistance of all the members of the Franklin lab. Lastly, this research would not have been possible had it not been for the support of VCU's Rice Center for Environmental Life Sciences.

Table of Contents

	Page
List of Tables.....	iv
List of Figures.....	v
Abstract.....	vi
Introduction.....	1
Methods and Materials.....	5
Results.....	14
Discussion.....	24
Conclusions.....	35
Literature Cited.....	36
Appendix.....	41
Vita.....	64

List of Tables

Table 1: Reagents and conditions for extracellular enzyme assay.....	41
Table 2: Plant species richness for each Site by month.....	42
Table 3: Two way ANOVA with means and standard error for soil properties.....	43
Table 4: Spearman Rank correlations of environmental variables and extracellular enzymes....	44
Table 5: Two way ANOVA with means and standard error for extracellular enzymes.....	45
Table 6: Two way ANOVA for leucyl aminopeptidase by month with means and SE.....	46
Table 7: Mantel Tests.....	47

List of Figures

Figure 1: Experimental design.....	48
Figure 2: Plant community structure (NMDS)	49
Figure 3: Below-ground and above-ground biomass for Vegetated and Clipped.....	50
Figure 4: C:N, soil organic matter, saturation for Vegetated and Clipped Treatments.....	51
Figure 5: Hydrologic regime at each Site for duration of study	52
Figure 6: Treatments pH, redox, and temperature for Vegetated and Clipped treatments.....	53
Figure 7: Environmental variables Principal Components Analysis (PCA).....	54
Figure 8: Extracellular enzyme analysis of β -1,4 glucosidase	55
Figure 9: Extracellular enzyme analysis of 1,4- β – cellobiosidase	56
Figure 10: Extracellular enzyme analysis of β -D-xylosidase.....	57
Figure 11: Extracellular enzyme analysis of Phenol oxidase.....	58
Figure 12: Extracellular enzyme analysis of Arylsulfatase.....	59
Figure 13: Extracellular enzyme analysis of Alkaline phosphatase.....	60
Figure 14: Extracellular enzyme analysis of Leucyl aminopeptidase.....	61
Figure 15: Extracellular enzyme Principal Components Analysis (PCA).....	62
Figure 16: Redundancy Analysis (RDA).....	63

Abstract

MICROBIAL COMMUNITY FUNCTION IN FRESHWATER WETLAND SOILS: USING EXTRACELLULAR ENZYME ANALYSIS TO STUDY THE EFFECT OF MOISTURE AND VEGETATION

By Aaron J. Porter, M.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
in Environmental Studies at Virginia Commonwealth University

Virginia Commonwealth University, 2011

Major Director: Rima B. Franklin, Assistant Professor, VCU Department of Biology

Differences in microbial community function via extracellular enzyme activity (EEA) were investigated to determine the effects of hydrology and plant-soil-microbe interactions in a young non-tidal freshwater riparian wetland. This was accomplished by establishing three plots along a moisture gradient at the VCU Rice Center; within each, five subplots were undisturbed while another five were cleared of all above-ground plant biomass each week for two years. Every eight weeks, soil cores (top 10 cm) were analyzed for pH, redox, carbon to nitrogen ratio, organic matter content, saturation, and temperature. Simultaneous assessment of microbial EEA revealed a site difference due to soil moisture content, which had an effect on soil pH, redox potential, and plant community composition. For most extracellular enzymes, the presence of vegetation was associated with higher activity. Together, the findings highly the importance of reestablishing native hydrologic and vegetated conditions in order to achieving previous functionality during wetland restoration.

Introduction

Wetlands are one of the most productive ecosystems on earth. Wetlands are often described as “the kidneys of the landscape” because they function as the receivers of waste and water from both natural and human sources (Kuehn, 1998; Mitsch and Gosselink, 2000). Wetlands provide habitat for numerous plant and animal species, and function to mitigate floods, recharge aquifers, improve water quality, and cycle nitrogen, carbon, phosphorous, and sulfur (Mitsch and Gosselink, 2000). Wetland ecosystems make up about 6% of the earth’s land surface, yet they contain 68% of soil carbon reserves (Mitsch and Gosselink, 2000; Reddy and DeLaune, 2008). Unfortunately, half of the original wetlands on earth have been lost due to population expansion, over harvesting, hydrologic modification, pollution, coastal development, and legal policies that have encouraged the widespread destruction of wetland habitats (Mitsch and Gosselink, 2000).

The United States has had a “no net loss” wetlands policy since President George H. Bush put the concept into law in 1989. This policy does not prevent the destruction of existing ecosystems but instead promotes the construction of new, or the restoration of formerly altered, wetlands to replace those that had previously been dammed or drained (Blankenship, 1994; Mitsch and Gosselink, 2000). Unfortunately, replacing wetlands, particularly non-tidal wetlands, with their complex hydrological and ecological linkages, is difficult. These ecosystems are characterized by hydric soils that support hydrophilic vegetation (Mentzer et al., 2006), and

though most restored wetlands may support these plants, many scientists agree they often don't perform all the chemical and habitat functions of a "natural" wetland (Blankenship, 1994).

In the past, the success of restoration projects has been judged on above-ground indicators such as plant and wildlife diversity; below-ground activity, which dictates most biogeochemical cycling, is understudied or outright ignored. Of particular interest are the microbial communities found in the soil of wetland ecosystems as they play a critical role in regulating the cycling, retention, and release of major nutrients and soil carbon (Hartman et al., 2008). Soil microbes represent the majority of the number of organisms in soil, and contain a large portion of the earth's genetic diversity; it has been estimated that one gram of soil contains as many as $10^{10} - 10^{11}$ bacteria (Whitman, et al., 1998; Homer-Devine et al., 2003). On the rare occasions that microbial attributes have been considered as part of wetland restoration projects, significant differences have been found. For example, a study conducted in 2010 by Card and Quideau reported that there were significantly lower levels of microbial biomass in the soils of newly restored wetlands (1 to 6 years old) compared to older systems (7 to 11 years post restoration). When the older restored wetlands were compared to reference sites that had never been altered, there were significantly higher levels of microbial biomass in the reference sites (Card and Quideau, 2010).

The microbial and plant communities in wetland soils can compete for resources and/or work symbiotically with one another in the conversion of organic and inorganic compounds to biologically available nutrients (Reddy and DeLaune, 2008). It has been well documented that the density and composition of the soil microbial community can be affected by the plant species and genotypes growing in that soil (Bever et al., 1997). For example, Zak et al. (2003) found that differences in plant diversity can impact ecosystem processes in wetlands by modifying the

composition and function of heterotrophic microbial communities in the soil profile. This decrease in microbial processing rates reduces the nutrients available to both above and below-ground plant biomass, and creates a feedback loop between the plants and microorganisms. The composition of the soil microbial community can have large differential effects on plant species richness, abundance, primary productivity, nutrient flow, and nitrogen fixation (Bever et al., 1997; Gutknecht et al., 2006). This often occurs via root-soil interactions that affect a number of geochemical characteristics in the rhizosphere including the concentration and availability of electron acceptors, dissolved organic carbon quality, and nutrient/contaminant uptake (Windham-Myers et al., 2009).

Hydrology is generally considered to be the dominant force structuring wetland plant communities. The hydrological conditions in wetlands can support a unique composition of vegetation and can either limit or enhance plant species richness (Mitsch and Gosselink, 2000). Primary productivity in wetlands is often improved by flowing and depressed by stagnant conditions due to restricted soil aeration resulting in reduced soils (Reddy and DeLaune, 2008; Mitsch and Gosselink, 2000; Bossio, 2006; Bossio et al., 2006). Increased water levels produce anaerobic conditions, which lowers the rate of microbial decomposition, resulting in the accumulation of carbon and nutrient rich organic matter. The presence of both aerobic and anaerobic conditions in wetland soils supports a diverse array of microbes that likely differ from those found in unsaturated soils (Bever et al., 1997), Bossio et al. (2006) found that flooding and the associated changes in aeration are the primary determinants of soil microbial community composition. The presence of plants usually increases microbial process rates in wetland ecosystems. For example, plants can influence soil microbial activities directly via root exudates that include labile carbon compounds, which can be converted into energy for microbial

respiration and biomass accumulation (Reddy and DeLaune, 2008). Plants release oxygen and organic carbon into the rhizosphere resulting in enhanced redox potential and nutrient substrate availability. Despite this knowledge, the link between above and below-ground community structure has yet to be fully understood (Gutknecht et al., 2006).

Traditionally, above and below-ground biota have been studied in isolation of one another. Recently, increased recognition of the role of above-ground-- below-ground feedbacks on ecosystem functions and properties has begun. Plants provide both the organic carbon needed for decomposer subsystems and numerous resources for obligate root-associated organisms (Mitsch and Gosselink, 2000). The decomposer subsystem breaks down dead plant material and indirectly provides soil nutrients for primary productivity. Similarly, root-associated microorganisms and their consumers have a direct influence on the quality, direction, and flow of energy and nutrients between plants and decomposers (Wardle et al., 2004; Wiessner et al., 2007). When available substrate becomes limited, soil microbes excrete extracellular enzymes to break down complex compounds to the degree that they can be absorbed by both the plant, for primary production, and the microbial community, for respiration and biomass accumulation. This study will seek to increase understanding of plant-soil-microbe relationships in a young riparian wetland along a moisture gradient.

Materials and Methods

1. Site Description

Research was conducted in the newly exposed upper basin of former Lake Charles, located along the James River in Charles City County, Virginia, on the property of Virginia Commonwealth University's Inger and Walter Rice Center for Environmental Life Sciences (Figure 1). In 1928, a dam was constructed at the edge of the property bordering the James River. The cypress-dominated wetland was clear-cut; the basin was then turned into a lake by King Fulton for use as a hunting and fishing club. In 2006, a heavy rain event caused a breach in the dam that subsequently drained the basin. The basin has been recolonized over the past four years with emergent wetland vegetation. The majority of the basin is tidally influenced; however, the northern end, where this research was conducted, remains non-tidal. The three research sites used in this study are predominantly groundwater fed, but also receive inputs from precipitation and occasional surface flow deposited in the basin during storm events from upland areas. Ongoing monitoring in the research basin has shown that plant production is consistent with freshwater marshes nearby (Gillespie and Franklin, in preparation), and that saturated anaerobic conditions are maintained in the soil (Jenkins, 2010).

2. Experimental Design

In March 2009, Christine Prasse established 7.5 m x 7.5 m plots within three distinct hydrological regions of the wetland as part of her M.S. research (Prasse, 2010). These three sites

have been maintained and are being used in this study of a second consecutive growing season (Figure 1). The “Wet Site” (37.3348 N, -77.2085 W) was established in an area that is permanently inundated above the soil surface and dominated by obligate wetland plants. The “Intermediate Site” (37.3358 N, -77.2079 W) is characterized by inundation above the soil surface, but does undergo dry-wet cycles. Conversely, the “Dry Site” (37.3369 N, -77.2068 W) is not typically inundated above the soil surface; however, the soil remains saturated within the rooting zone.

Each of the three main plots was subdivided into 25 subplots (1.25 m x 1.25 m). Within this grid, ten subplots were randomly selected and cleared of all above-ground plant material as well as the first five cm of the surface organic matter layer (to minimize the seed bank as per Zak et al., 2003). During the first growing season, vegetation was allowed to re-grow in five subplots in each main plot; these plots were designated as “Vegetated”. Vegetation grew throughout the first growing season, senesced during the fall and winter, and then was allowed to continue growing throughout the entire second growing season. The other five subplots in each main plot were surrounded by construction fencing to keep neighboring vegetation from encroaching upon them, and designated “Clipped.” They were weeded on a weekly basis throughout both growing seasons (April – November); subplot perimeters were simultaneously cut using a straightedge shovel (30 cm deep) to reduce root intrusion. Subplot maintenance was not carried out during the week prior to each sampling date to minimize soil disturbance. The sampling considered for this thesis began in May 2010 and continued every eight weeks with subsequent sampling dates in July, September, and November.

3. *Sampling*

In order to reduce edge effects from adjacent vegetation, all samples were taken in the 1 m x 1 m center of each subplot; overlap with prior sampling locations, including the first year of this study, were avoided. Within this area, a 25 cm by 25 cm square made of PVC piping was used to randomly select each sample area. Soil temperature (°C) was recorded, and then above-ground plant biomass was clipped to the soil surface and packaged for analysis. Using a 10-cm long hand shovel, a 5 cm wide x 10 cm deep soil sample was collected. To measure below-ground plant biomass, a de-tipped 60-ml syringe was used to pull soil cores from the sample area. All samples were then placed in appropriately labeled plastic bags and homogenized on site. Samples were stored at ambient temperature for return to the lab. All samples were refrigerated in the lab within two hours of collection. Inside the 7.5 m x 7.5 m, perimeter of each main plot, all species of vegetation were observed and recorded. Ecotone piezometers (Acceeca, Christchurch, New Zealand) were installed at the center of each main plot and programmed to record daily groundwater levels (4:00 AM). Data was pulled from each well on every sampling date using a hand held Aceeca MEZ1000 Meazura. Each data point was used to plot hydrographs for each site using Palm Desktop Pro version 4.1. and Sigma Plot version 10.0.

Upon return to the lab, soil samples were immediately measured for redox and pH using a HANNA Combo pH and ORP probe (HANNA instruments®, Inc., Woonsocket, Rhode Island). Subsamples were taken for soil characterization as described below. The remaining soil was stored at 4°C for enzyme analysis, which was conducted within ten days of the sampling date as per Marx et al., 2001.

4. Soil Characterization

To determine gravimetric moisture content, a subsample of soil from each subplot (10-25 g wet weight) was dried in aluminum boats at 95°C for 72 H. The dry soil was combusted at 425°C for 24 H in an Isotemp Programmable Muffle Furnace (Fisher Scientific, Pittsburgh, Pennsylvania) to determine soil organic matter (SOM) content as loss on ignition. A separate subsample was archived at -20°C until it could be analyzed for soil carbon to nitrogen content; following acidification and grinding, the sample was analyzed using the Series II CHNS/O Analyzer 2400 (PerkinElmer, San Jose, California) by the VCU Environmental Analysis Lab.

5. Vegetation

The plant material harvested from each subplot was dried at 70°C for 7 days to estimate above-ground biomass (kg dry weight per m²). Root biomass was determined by submerging each 60 ml soil core in 100 ml deionized (DI) water for 48 hours. The softened soil was strained through a 0.45 µm sieve. Roots were removed from the sieve using small tweezers, rinsed by hand, and dried at 65°C for 48 H. Root biomass was calculated at mg dry root per cm³.

6. Soil Microbial Function Analysis

In this research, extracellular enzyme activity (EEA) was measured by spiking soils with fluorescently-labeled substrates. Enzymatic breakdown of these substrates releases the fluorescent label and the amount of light detected is proportional to the amount of enzymes in the original sample. Six hydrolytic substrates were selected that are associated with labile carbon (β-1,4 glucosidase and 1,4- β – cellobiosidase), hemicellulose recalcitrant carbon (β-D-xylosidase), nitrogen (leucyl aminopeptidase), phosphorous (alkaline phosphatase), and sulfate

(arylsulfatase). One oxidoreductase enzyme, phenol oxidase, was chosen for its association with the breakdown of recalcitrant carbon compounds and analyzed using a colorimetric assay.

Assays were completed within ten days of field sample collection. All substrates, standards, and buffers were obtained from Sigma-Aldrich Co, Ltd (Saint Louis, Missouri). All measurements were made using BioTek Synergy II microplate reader and Gen5 software version 1.07.

6.1 Substrate, Buffer, and Standards Preparation

Each substrate was prepared fresh, using the appropriate solvent and protocol from Marx et al. (2001). Depending on the substrate being tested, either 4-methylumbelliferone (MUB) (# M1381) or 7-amino-4-methylcoumarin (AMC) (# A9891) standards were prepared for a quench assay (Table 1). Quench range (pmol) varied between each substrate tested; therefore a separate quench range correction procedure was applied for each substrate (Marx et al., 2007). A 10-mM stock solution of pure standard MUB was prepared in methanol, then a serial dilution was performed using 0.1 M MES buffer (2-[N-Morpholio]ethanesulfonic acid, pH 6.1) until attaining a 2 mM concentration. A 20 mM AMC stock was dissolved in methanol and then diluted to 2 mM using 0.05 M Trizma buffer (pH 7.8). Concentrated standards were prepared and then stored in the dark at 4 °C for up to 1 week; diluted standards were prepared fresh daily. A 50-mM sodium bicarbonate buffer (pH 6.1) was used for the colorimetric phenol oxidase assay.

6.2 Microtiter Plate Setup

6.2.1. Soil Preparation

Soil was prepared by adding 1.0 g soil to 100 mL DI water. The mixture was sonicated at 15 W for 2 minutes using the Misonix Sonicator 3000. The soil suspension was then placed on a

shaker table and rotated at 150 rpm as 50 µl aliquots were dispensed into the appropriate wells for each assay.

6.2.2. *Fluorescent Assay*

For each soil sample, three enzyme assay replicates were prepared as well as eight quench standards. Enzyme assays contained a sample, buffer, and substrate; meanwhile, quench standards contained sample, buffer, and varying amounts of MUB or AMC (Table 1). On each plate, one column was reserved for a negative control that contained 50 µl of sterile DI water, substrate, and buffer. The final volume in each well was set to 200 µl (Marx et al., 2007). All plates were then slowly rotated in a 30⁰ C dark incubator until being read on the Biosynergy II (excitation 360 nm and emission at 460 nm); total reaction times for each substrate are summarized in Table 1.

6.2.3. *Colorimetric Assay (Phenol Oxidase EEA only)*

Three replicates of a negative sample control (50 µl sample + 100 µl of 50 mM NaHCO₃ buffer + 50 µl DI water), negative substrate control (50 µl sterile DI water + 100 µl of 50 mM NaHCO₃ buffer + 50 µl L-DOPA), and enzyme assay (50 µl sample + 100 µl of 50 mM NaHCO₃ buffer + 50 µl L-DOPA) were prepared for each soil sample. Plates were slowly rotated in a dark incubator at 20⁰C and then read at 410 nm using Biosynergy II. See Table 1 for total reaction time.

6.3 Calculations

Prior to calculating enzyme activity ($\mu\text{mol activity g dry}^{-1} \text{ soil H}^{-1}$), triplicates measurements were analyzed for experimental error using the coefficient of variation (CV). Any data with a CV greater than 10% was examined for outliers; when appropriate, a single outlier was removed. At least two data points were always kept for each sample for further analysis.

For fluorescent assays, enzyme activity was calculated using the equations provided by Steinweg and McMahon (2007) except that the coefficient of determination (R^2) for each quench correction was examined for experimental error; outliers were removed when a score of less than 0.95 was observed. Colorimetric-based phenol oxidase activity was calculated using a modified equation provided by Sinsabaugh (2009). Optical density was computed by subtracting the negative sample control from the negative substrate control and background control. The micromolar extinction coefficient of 0.48 per μmol was empirically determined at 410 nm.

7. Statistical Analysis

7.1 ANOVA

Measurements are summarized with F statistics, p-values, means, and standard errors for each Treatment (C or V) and site (Wet, Intermediate, Dry) combination. A two-way ANOVA model was run with site and treatment main effects and a site x treatment interaction. In preliminary analysis several ANOVAs were conducted with Time as a main effect; little consistency was observed, therefore data was pooled. Significant differences were interpreted based on $\alpha = 0.05$. All ANOVA tests were run in PAST version 2.07 (Hammer et al., 2005).

7.2. Principal Component Analysis (PCA)

To compliment the individual ANOVA assessment, a multivariate approach was utilized to achieve a better understanding of overall patterns in the data. A PCA was conducted combining all environmental variables. A separate PCA was conducted combining all EEA data. All variables were combined in a correlation matrix; the analysis was conducted in PAST v. 2.01.

7.3. Correlation of Environmental and Biological Variables

A correlation analysis was conducted using Spearman's rank order coefficient (r_s) and the PAST statistical package version 2.01 to quantify the association between each pair of soil environmental properties and EEA.

7.4. Mantel Tests

In order to analyze the relationship between plant species composition against all environmental data and EEA, a series of Mantel tests were conducted using PAST version 2.01. A data matrix was formed from the presence/absence data for plant species composition by using Dice's similarity coefficient in order to place double weight on any species presence, and to avoid inferring similarity based on plant species absence (Legendre and Legendre, 1998). The data matrices for both EEA and the environmental parameters was created using Gower's coefficient of dissimilarity. A Monte-Carlo permutation test ($N = 500$) was conducted to test significance. Significance levels were based on $\alpha = 0.05$ and Bonferroni corrected for multiple

comparisons. To compliment the Mantel tests, a Non-metric Multidimensional Scaling (NMDS) plot was developed to examine site and month effects on plant community composition.

7.5. Redundancy Analysis (RDA)

Redundancy Analysis (RDA) was conducted using CANOCO v. 4.5 to examine overall patterns for each set of EEA results, soil properties, and above-ground biomass. RDA is similar to Canonical Correspondence Analysis (CCA) and is a method for extending a multiple regression that has a single response variable (Y) and multiple predictor variables (X) to a multiple regression involving multiple response variables (Y) and a common matrix of predictor variables (X) (Peres-Neto, et al., 2006). The method was originally described by Rao (1964) as a multivariate statistical model well suited for ecological studies. Redundancy refers to quantity, and is defined as the proportion of the total variance of a measurement domain predictable from a linear composite of the other domains (Legendre and Anderson, 1999). Predictor variables consisted of pH, redox, soil saturation, SOM, C:N, below-ground root biomass, and above-ground biomass; explanatory variables (designated as “species” in CANOCO v 4.5) consisted of all EEA data. Scaling was focused on “inter-species correlations”, species scores were divided by the standard deviation, species data was centered and standardized, and a Monte-Carlo permutation test (N = 5000) determined the significance of the first axes, as well as the significance of all canonical axes together. Two RDAs were run in order to isolate the effect of Treatment (Vegetated vs Clipped).

Results

An ANOVA approach was used to assess mean significant differences and interactions by site and treatment for all soil, vegetation, and EEA data. First, each sampling event (May, July, September, and November) was analyzed separately for each parameter using a two-way ANOVA. In nearly all cases, the patterns were consistent across all four sampling events; therefore, data was pooled and analyzed using a two-way ANOVA. The sole exception to this was the EEA for leucyl aminopeptidase, which showed a consistent temporal pattern; each month was thus assessed independently. For all two-way ANOVAs, whenever a significant site x treatment interaction occurred a series of one-way ANOVAs were run to further examine the interaction. Above-ground plant biomass data only contained values for Vegetated Treatments; as a result, this data only required analysis via one-way ANOVA.

1. *Vegetation Biomass and Plant Community Composition*

NDMS analysis showed these three sites differed based on the presence/absence of each species in each plot (Sorenson's index stress = 0.12) (Figure 2). Ten species were common across all three sites (Table 2): *Agrostis sp.*, *Boehmeria cylindrica*, *Commelina communis*, *Hibiscus moscheutos*, *Juncus effusus*, *Leersia oryzoides*, *Ludwigia palustris*, *Murdannia keisak*, *Polygonum sagittatum*, and *Saccharum alopecuroides*. Species richness was highest during July and September and lowest in May and November. The Dry Site had the greatest richness due to

conditions that were favorable not only to obligate wetland plants, but also wetland and upland facultative species.

An ANOVA was used to compare above-ground biomass (kg dry plant weight per m²) across sites using data from the Vegetated Treatments (Figure 3, C). Site had a significant effect ($F=3.53$, $p=0.036$) with higher plant biomass at the Wet (1.8, SE = 0.2) and Intermediate (2.1, SE = 0.4) sites compared to the Dry Site (1.1, SE = 0.2). For below-ground biomass (mg dry root weight per m³), there was not a significant site x treatment effect or individual site effect, however a significant Treatment effect ($p < 0.001$) was observed (Figure 3, A, B; Table 3). Mean below-ground biomass was consistently higher at the Vegetated Treatments (0.014, SE = 0.001) than the Clipped Treatments (0.004, SE = 0.001).

2. Site and Moisture Effects

Soil saturation (gravimetric moisture content (%)) was consistently higher at the Wet and Intermediate sites compared to the and Dry Site; Vegetated Treatments were higher than Clipped Treatments (Figure 4, E,F; Table 3). No significant site x treatment interaction was present ($p = 0.2198$); however, significant site ($p < 0.001$), and treatment ($p < 0.001$) effects were observed. Mean soil saturation was highest at the Wet Site (176, SE = 4), lower at the Intermediate Site (105, SE = 3), and lowest at the Dry Site (44, SE = 2). The Vegetated Treatments had a higher mean percent saturation (114, SE= 8) than the Clipped Treatments (102, SE = 7). Analysis of soil moisture content revealed that the experimental design was successfully positioned along a moisture gradient, and that the removal of vegetation consistently decreased soil moisture.

Piezometer well data was taken every day for the duration of the study (Figure 5). Results showed a very distinct difference in hydrology between the Wet and Dry sites, as well as the Intermediate and Dry sites. Contrary to soil saturation data, the piezometer well data revealed minimal differences between Wet and Intermediate sites.

3. ANOVA Results for Soil Properties

Soil temperatures ($^{\circ}\text{C}$) followed seasonal air temperature (results not presented); average soil temperatures ranged from 20.2 ± 0.4 (May), 25.3 ± 0.1 (July), 18.3 ± 0.2 (September), 12.1 ± 0.1 (November) (Figure 6, E,F). There was no significant site x treatment interaction on soil temperature, nor were there any significant site or treatment differences (Table 3)

Soil pH was consistently highest at the Wet Site and Vegetated Treatments (Figure 6, A,B; Table 3). No significant site x treatment interaction was observed; however, a significant site effect ($p < 0.001$) was noted. Mean pH level at the Wet Site (5.7, SE = 0.1) was the least acidic among the three sites, the Intermediate Site was slightly more acidic (5.3, SE = 0.1), and the most acidic pH was found at the Dry Site (3.8, SE = 0.1). These results indicate that soil pH was most affected by site (soil moisture), rather than treatment (presence or absence of plants).

Redox potential (mV) was consistently greater at the Dry Site (459, SE = 8.1) compared to the Wet (-36.2, SE = 10.4) or Intermediate (27.5, SE = 21.3) sites (Figure 6, C,D; Table 3). No significant site x treatment interaction or treatment effect was observed; however, a significant site effect was noted ($p < 0.001$). As with pH, the lack of a significant difference based on treatment indicates that redox potential is primarily dependent on site.

Analysis of SOM (%) revealed a significant site x treatment interaction ($p = 0.0172$) (Figure 4, C, D; Table 3). A series of one-way ANOVAs were run to further examine this relationship. A site effect was detected in the Vegetated Treatments ($F = 12.04$, $p < 0.001$); however, this effect disappeared when vegetation was removed ($F = 2.70$, $p = 0.076$). SOM increased along the moisture gradient; the Wet Site had the highest SOM content (10.2, SE = 0.5), followed by the Intermediate Site (8.7, SE = 0.3), and then the Dry Site (7.0, SE = 0.5). Tukey's post hoc analysis revealed that mean values for the Dry Site were significantly different than all others (Dry-Wet: $p < 0.001$; Dry-Int.: $p = 0.021$); the Wet and Intermediate sites were not significantly different ($p = 0.068$).

Carbon to Nitrogen ratios (C:N) were computed to analyze SOM quality. A significant site x treatment interaction was observed for C:N ($p = 0.0077$) (Figure 4, A, B; Table 3). To further investigate this interaction, a series of one-way ANOVAs were run to isolate each distinct site and treatment effect. A significant site effect was found regardless of treatment; (Vegetated: $F = 5.6$, $p = 0.006$; Clipped: $F = 19.86$, $p < 0.001$). Tukey's post hoc analysis found C:N to be significantly higher at the Dry Site (13.2, SE = 0.3) than the Wet Site (12.1, SE = 0.2) in Vegetated Treatments ($p = 0.0057$). In Clipped Treatments, C:N values in the Dry Site (14.6, SE = 0.4) were significantly higher than both the Wet Site (12.1, SE = 0.3) ($p < 0.001$) and the Intermediate Site (12.1, SE = 0.2) ($p < 0.001$).

4. PCA of Soil and Plant Data

In order to assess trends across multiple parameters soil moisture, pH, redox, C:N, SOM, above-ground biomass, and below-ground biomass were combined in a principal component analysis (Figure 7). Soil temperature was excluded from the analysis because it showed no

significant treatment or site effects, and only induced a temporal effect when included. The first axis of the PCA, with high factor loadings from redox (-0.94), pH (0.89), and soil saturation (0.88), explained 43.2% of the variance in the data. The second axis, with high factors loadings from below-ground (0.84) and above-ground (0.74) biomass, explained 19.1% of the variance in the data. All other loadings associated with this PCA were less than 0.64.

5. *Spearman Rank Correlation (r_s)*

Relationships among soil, vegetation, and EEA were examined using a Spearman Rank correlation matrix (Table 4). The analysis revealed a considerable amount of interrelation among variables. Saturation was found to have a particularly strong relationship, and was significantly correlated with all the other soil, vegetation, and EEA data. Redox, pH, SOM, C:N, below-ground biomass, and above-ground biomass were found to be closely related to each other as well as EEA data. For a complete list of correlation values see Table 4.

6. *Extracellular Enzyme Function (EEA)*

6.1. *Labile C Substrates*

β -1,4 glucosidase activity ($\mu\text{mol activity g dry}^{-1} \text{ soil H}^{-1}$) (Figure 8, Table 5) was significantly different across sites ($p < 0.001$) and between treatments ($p < 0.001$), though no significant site x treatment interaction was observed. Activity was significantly higher at the Wet Site (0.271, SE = 0.021), than the Intermediate Site (0.179, SE = 0.017) and Dry Site (0.131, SE = 0.016). Activity was consistently higher in the Vegetated Treatments (0.258, SE = 0.016) than the Clipped Treatments (0.129, SE = 0.012).

EEA of 1,4- β – cellobiosidase ($\mu\text{mol activity g dry}^{-1} \text{ soil H}^{-1}$) (Figure 9, Table 5) was significantly different across sites ($p < 0.001$), however no significant site x treatment interaction or treatment effect was observed. Activity was significantly higher at the Wet Site (0.941, SE = 0.036), than the Intermediate (0.691, SE = 0.024) and Dry sites (0.434, SE = 0.016).

6.2. *Recalcitrant C Substrates*

β -D-xylosidase activity ($\mu\text{mol activity g dry}^{-1} \text{ soil H}^{-1}$) (Figure 10, Table 5) was significantly different across sites ($p < 0.001$) and between treatments ($p < 0.001$), however no significant site x treatment interaction was observed. Similar to the EEA for the more labile substrates, activity was significantly higher at the Wet Site (0.498, SE = 0.041), than the Intermediate Site (0.358, SE = 0.034) and Dry Site (0.316, SE = 0.031). Activity was higher in the Vegetated Treatments (0.461, SE = 0.034) than the Clipped Treatments (0.320, SE = 0.026).

Phenol oxidase activity ($\mu\text{mol activity g dry}^{-1} \text{ soil H}^{-1}$) (Figure 11, Table 5) was the only EEA that revealed a significant site x treatment interaction. To further investigate this interaction, a series of one-way ANOVAs were conducted. Site had a significant effect on EEA regardless of treatment (Vegetated: $F = 3.53$, $p = 0.036$; Clipped: ($F = 16.13$, $p < 0.001$). In Vegetated Treatments, Tukey's post hoc analysis revealed that mean EEA rates in the Dry Site (2.110, SE = 0.343) were significantly higher than in the Wet Site (0.965, SE = 0.288) ($p = 0.0294$). In Clipped Treatments, activity was significantly higher ($p < 0.001$) in the Dry Site (4.097, SE = 0.459) than the Wet (1.628, SE = 0.413) and Intermediate sites (1.348, SE = 0.211).

6.3. *S Substrate*

Arylsulfatase ($\mu\text{mol activity g dry}^{-1} \text{ soil H}^{-1}$) (Figure 12, Table 5) was significantly different across sites ($p < 0.001$) and between treatments ($p < 0.001$); however, no significant site x treatment interaction was observed. Activity was significantly higher at the Wet Site (0.254, SE = 0.019), than the Intermediate Site (0.195, SE = 0.016) and Dry Site (0.121, SE = 0.009). Activity was higher in the Vegetated Treatments (0.228, SE = 0.015) than the Clipped Treatments (0.152, SE = 0.012).

6.4. *P Substrate*

Alkaline phosphatase ($\mu\text{mol activity g dry}^{-1} \text{ soil H}^{-1}$) (Figure 13, Table 5) was significantly different across sites ($p < 0.001$) and between treatments ($p < 0.001$); however, no significant site x treatment interaction was observed. Activity was significantly higher at the Wet Site (3.442, SE = 0.174), than the Intermediate Site (2.590, SE = 0.169) and Dry Site (2.433, SE = 0.180). Activity was higher in the Vegetated Treatments (3.292, SE = 0.153) than the Clipped Treatments (2.351, SE = 0.126).

6.5. *N Substrate*

Leucyl aminopeptidase activity (Figure 14, Table 6), unlike the other extracellular enzymes studied in this research, was not consistent across all sampling events; therefore, each month was analyzed via separate two-way ANOVA. A significant site x treatment effect ($p < 0.001$) was observed in May, July, and September. However, this effect disappeared in November indicating a possible relationship with plant senescence. Mean EEA was always highest,

regardless of month, in Wet-Vegetated Sites and lowest in Dry-Clipped Sites (see Table 6 for all means and standard errors).

7. *PCA of All Extracellular Enzyme Assays*

In order to assess trends across EEA, all extracellular enzymes were combined in a principal component analysis (Figure 15). The first axis of the PCA explained 42.9% of the variance in the data; with high factor loadings on β -1,4 glucosidase (0.83), alkaline phosphatase (0.75), arylsulfatase (0.74), and 1,4- β – cellobiosidase (0.72). All other factor loadings were less than 0.52. The second axis of the PCA explained 18.3% of the variance in the data. The only high factor loading was for leucyl aminopeptidase (0.68); all other factor loadings were less than 0.47. Unlike the PCA of the environmental data (Figure 7), which showed consistent site (PC 1) and treatment (PC 2) effects, the PCA of the EEA groups samples by site only (Figure 15). Activity increased from left to right along the moisture gradient. At the far left, we find EEA samples associated with the lowest soil moisture content (e.g., Clipped-Dry = 42, SE = 3), and at the far right we find those associated with the highest soil moisture content (e.g., Vegetated-Wet = 182, SE = 7).

7.1. *Redundancy Analysis (RDA)*

RDA was used to examine the relationship between all environmental parameters (excluding temperature) and EEA (Figure 16; A, B). In these biplots, the length of arrows associated with explanatory variables indicates relative impact on EEA. The cosine between explanatory and response arrows indicates correlation strength; explanatory variables positioned at or near a right angle to a given response variable indicates a correlation of approximately 0, while explanatory

variables positioned at or near 180 degrees of any given response variable indicates a strong negative correlation. Two RDA analysis were conducted to isolate the effect of environmental parameters on EEA in plots characterized by the presence of above-ground biomass (Figure 16, A) and in the absence of above-ground biomass (Figure 16, B). A Monte-Carlo permutation test ($N = 5000$) was used to test the significance of all canonical axis in vegetated treatments ($F = 4.32$, $p = 0.002$). Eigenvalues were computed for each canonical axis to show the total amount of variance explained by the model: axis 1 = 0.256, axis 2 = 0.062, axis 3 = 0.037, and axis 4 = 0.007. The sum of all eigenvalues was 0.368, indicating that approximately 37% of variance was explained by the RDA correlation biplot. The significance of all canonical axis was then tested in clipped treatments ($F = 6.47$, $p = 0.002$). Again, eigenvalues were computed for each canonical axis to show the total amount of variance explained by the model: axis 1 = 0.279, axis 2 = 0.079, axis 3 = 0.047, and axis 4 = 0.011. The sum of all eigenvalues was 0.423, indicating that approximately 42% of variance was explained by this RDA correlation biplot. All four eigenvalues reported are canonical and correspond to axes that are constrained by the environmental variables.

8. *Mantel Correlations (r_m)*

Because the composition of the plant community was recorded as binary data (presence or absence of each species in each main plot), it was not possible to analyze its relationship to EEA using the typical correlation/regression approach that forms the foundation of PCA and RDA. Instead, a series of Mantel tests were conducted to compare similarity in plant community composition to similarity in EEA (Table 7). Soil properties were also compared to plant community composition to investigate potential indirect effects on EEA. Changes in plant

community composition were significantly positively correlated with arylsulfatase activity ($r_m = 0.54$), and to the activity of both labile carbon-related enzymes (β -1,4 glucosidase: $r_m = 0.35$; 1,4- β – cellobiosidase: $r_m = 0.54$).

All soil environmental variables were combined in a matrix using Gower's coefficient of dissimilarity and compared to plant community composition ($r_m = 0.44$). Each variables was then compared to species composition individually, soil moisture proved to be most strongly correlated ($r_m = 0.78$), followed by redox ($r_m = 0.65$), SOM ($r_m = 0.47$), and pH ($r_m = 0.46$).

Discussion

Wetlands are a critical element of the global landscape due to their unique capability to regulate biogeochemical cycles by utilizing microbial communities adapted to life in hydric soils. This study aimed to quantify how microbial community function, via EEA, responds to the effects of hydrology and the presence or absence of vegetation in a young non-tidal riparian wetland. Enzymes are the main mediators of soil biological processes; including OM decomposition, mineralization, and nutrient cycling (Marx et al., 2001). Hydrolytic enzymes control the rate at which substrates are degraded and become available for microbial or plant uptake by catalyzing the cleavage of bonds through the addition of water (Reddy and DeLaune, 2008). Oxidoreductases are a second form of enzymes responsible for catalyzing oxidation-reduction reactions (Reddy and DeLaune, 2008). Enzyme diversity and associated activities provide a useful method to examine functional diversity in soils.

Changes in hydrology and vegetation have both direct and indirect effects on community function. By selecting three sites along a moisture gradient and clearing all vegetation from select subplots, it was possible to observe the effects of environmental conditions on extracellular enzyme rates in situations where soil moisture and vegetation conditions differed. This discussion examines the effect of vegetation and saturation on the soil environment, and then examined relationships between soil properties and microbial community function (EEA).

1. Saturation and Vegetation Effects on Soil Parameters

The results of this study indicate significant differences in soil saturation among all three main sites (Wet, Intermediate, Dry, Figure 4, E, F); however, daily groundwater levels show little difference in the hydrological regime between the Wet and Intermediate sites (Figure 12). Varied cross-site soil composition and/or differences in elevation could explain this contradiction. All three sites were classified as loam and had low clay content ($< 15\%$). The Dry Site was comprised mostly of sand (60%) and the Intermediate and Wet sites contained large and similar amounts of silt (~55%) (hydrometer method, A&L Laboratories, Richmond, Virginia). Despite the fact that all the sites originated from the same watershed and lithology, and thus were expected to be similar, variations in soil texture between sites formed as the basin drained following the breach in the dam. Sand rapidly settled in the north end of the basin, where the Dry Site is located, due to its characteristically large particle size. As the water moved from north to south towards the James River, silt particles began to settle; finally, clay, which is characterized by smaller particle size than sand or silt, settled out in greater abundance along a southern gradient, where the Wet Site is located. While groundwater measurements are informative, it appears they do not reflect well the soil conditions that the bacteria are likely to experience, and thus soil moisture content was tested as the predictor of microbial function in this study.

Variations in soil saturation resulted in strong differences in soil physiochemical properties and plant community composition (Figures 4, 6, and 2 respectively). Plant community composition at the Dry Site consisted of a range of obligate wet, facultative wet and upland plants. Composition at the Wet and Intermediate sites was restricted to hydrophilic vegetation (obligate and facultative wetland species) with adaptations for surviving in anoxic soils (Table

2). Soil moisture was strongly correlated to plant community composition ($r_m = 0.78$, $p < 0.001$), as were several physiochemical properties like pH ($r_m = 0.47$, $p = 0.0022$) and redox ($r_m = 0.65$, $p < 0.001$). To determine the independent impact of pH, redox, and soil moisture on plant species composition, a partial Mantel test was performed. Soil moisture was found to be the main driver of plant community composition ($r_m = 0.53$, $p = 0.0018$), and the pH or redox effects were deemed spurious ($r_m = 0.14$, $p = 0.1718$).

Due to the small spatial scale of the research (70 acres), it is reasonable to assume that the same lithology contributed water, soil, and sediment inputs at all three sites therefore saturation is likely the main driver of differences in pH and redox potential across the moisture gradient. Greater soil saturation leads to a decrease in redox potential because of limited oxygen diffusion from the atmosphere into the soil; this creates optimal conditions for the reduction of iron, manganese, nitrate, and sulfate, resulting in an increase in soil pH in wetter areas (Reddy and DeLaune, 2008).

Soil saturation had a strong positive relationship with SOM content ($r_s = 0.52$), and a significant negative correlation with the quality of SOM as measured by the ratio of Carbon to Nitrogen ($r_s = -0.51$). While these correlations are significant, the magnitude of the differences in SOM and C:N across the sites were small. For example, mean SOM content was only marginally higher at the Wet Site (10.6, SE = 7.9) than the Dry Site (9.7, SE = 1.4; Figure 4, B, C), and both are considerably lower than is typically seen in nearby freshwater wetlands (Gillespie, in preparation). Because the basin was only recently exposed, there has been little time for SOM to accumulate as it usually does in saturated wetland soils. The RDA biplot in Figure 16 illustrates the minimal effect of SOM; while the SOM vector correlates to several EEAs, the length of the vector is short, indicating a lack of influence due to low SOM content.

Conversely, the quality of the SOM (C:N) shows a strong negative correlation with several EEs, indicating that despite the low quantity of SOM, its quality affects EEA. Conversely, the quality of the SOM (C:N) shows a strong negative correlation with several EEs, indicating that despite the low quantity of SOM, its quality affects soil function. In general, lower C:N values are considered “higher quality” and net immobilization of nitrogen occurs when C:N ratio is greater than 20:1 (Reddy and DeLaune, 2008). C:N values for this study suggest relatively high-quality SOM, and, though the differences across sites was statistically significant, the range for this parameter was small (e.g., Wet Site mean = 12.1, Dry Site mean = 13.9). Sites with higher quality SOM (i.e., lower C:N ratio) consistently had higher levels of EEA.

When all environmental data, excluding temperature, were analyzed together via PCA, soil saturation, pH, and redox potential were the primary driving forces for observed variance among sites (Axis 1 in Figure 14); whereas roots and plant biomass were the driving forces behind treatment effects (Axis 2 in Figure 14). The absence of roots in Clipped Treatments during the second year (Table 5, Figure 4 (A,B)) of the study likely accounts for differences in EEA not observed in year one (Prasse, 2010). The data in this PCA plot are pooled across time (all months averaged for each site), and the low SE associated with each group further demonstrates that the temporal changes in soil properties were small compared to the site and treatment effects.

2. Extracellular Enzyme Function

2.1. C Substrates

Plant cell walls are the major reservoir of fixed carbon in nature, and have three main polymeric constituents: cellulose, hemicellulose and lignin (Tuncer, 2000). In this research, the

enzymes 1,4- β – cellobiosidase and β -1,4 glucosidase were studied for their ability to break down cellulose and similar labile carbon components of SOM (Sinsabaugh et al., 2008). Once broken down into biologically available forms, the microbial community can utilize that carbon for respiration and biomass accumulation (Sinsabaugh et al., 2008; Reddy and DeLaune, 2008).

For both of these enzymes, activity was highest at the wetter sites with anoxic conditions. These findings contradict popular consensus (Reddy and DeLaune, 2008), which predicts higher EEA rates under aerobic conditions. It is likely that EEA increased under anaerobic conditions due to sensitivity to pH, which was more alkaline under increased soil saturation, as well as higher levels of labile carbon in the plant litter that comprises the OM accumulation at the wetter sites (Reddy and DeLaune, 2008). β -1,4 glucosidase activity is specifically influenced by temperature (maximum activity at 30⁰C) and pH (maximum activity at 8.5) (Reddy and DeLaune, 2008).

A series of Mantel tests (Table 8) revealed a strong correlation between plant community composition and EEA for both of these cellulolytic enzymes. Thus differences in activity between hydrologically-distinct sites may be symptomatic of variance in plant community composition (Figure 2), instead or in addition to the pH effects described above. Regardless of treatment β -1,4 glucosidase exhibited a strong positive correlation to pH, SOM, and soil saturation; in Vegetated Treatments above-ground plant biomass was also strongly correlated to activity. A strong negative correlation between redox and EEA was present regardless of treatment; however, when vegetation was present a strong negative correlation was observed with C:N that did not exist in its absence (Figure 16; A, B). This was also true for 1,4- β – cellobiosidase activity. EEA was more severely affected by the quality of SOM in Vegetated Treatments due to a greater abundance of newly deposited SOM from plant detritus that occurred

during the two years of the study. Decomposition of new plant litter was restricted in Clipped Treatments due to the lack of above-ground vegetation. Regardless of treatment a positive correlation was observed between 1,4- β – cellobiosidase activity and soil saturation; however, below-ground biomass was strongly correlated only in the presence of vegetation (Figure 16; A, B). Over time, as SOM accumulates in greater quantities at the Wet Site, the difference in EEA between sites will likely increase due to increased availability of labile carbon in SOM and plant detritus. Similarly, any associated change in C:N is likely to shift EEA. Geisseler and Horwath, 2009 found C:N to be negatively correlated with β -1,4 glucosidase activity; a relationship that was confirmed by the results of this study (Figure 16, A).

As previously discussed, these cellulolytic enzymes are both responsible for breaking down cellulose; however only β -1,4 glucosidase showed significant differences across treatments. Its activity was higher in Vegetated Treatments for all sites along the moisture gradient (Table 5, Figures 8). In the presence of living plant biomass, soil microbes must compete for nutrients. Soil microbes excrete cellulolytic enzymes to increase the rate at which atmospheric carbon and labile carbon compounds in plant detritus and SOM become biologically available (Makoi and Ndakidemi, 2008). In the absence of vegetation, soil microbes lack competition for available carbon resources, resulting in lower EEA. For 1,4- β – cellobiosidase, no significant treatment effects were observed, and overall activity ($\sim 1 - 1.2 \mu\text{mol activity g dry}^{-1} \text{ soil H}^{-1}$) was much higher than β -1,4 glucosidase ($< 0.4 \mu\text{mol activity g dry}^{-1} \text{ soil H}^{-1}$) thus the response of β -1,4 glucosidase activity was more severely affected by differences in treatment.

β -D-xylosidase is responsible for hydrolyzing polymeric hemicelluloses into simple units such as xylan and xylobiose (Reddy and DeLaune, 2008). Like other hydrolytic enzymes in this study, EEA rates were consistently highest at the Wet Site and Vegetated Treatments (Figure 10,

Table 5). A Spearman rank correlation matrix found below-ground biomass (roots) to be the only strongly significant variable associated with β -D-xylosidase activity (Table 4); this relationship was confirmed in the RDA correlation biplot (Figure 16; A, B). The relationship between β -D-xylosidase and roots indicate a reliance on root ventilation for microbial respiration. Plant root ventilation provides aerobic microniches that aid this extracellular enzyme in the breakdown of hemicellulose compounds found in OM and plant detritus. Regardless of treatment EEA was positively correlated with soil saturation (Figure 16; A, B). Because hydrolytic enzymes catalyze the hydrolysis of a substrate through the addition of water, activity was further increased under wetter conditions. In the absence of above-ground vegetation pH was found to have a strong positive correlation to EEA; however, this relationship did not exist in Vegetated Treatments (Figure 16; A, B). Low-molecular-weight organic compounds in root exudates played a key role in increasing soil pH, which in Vegetated Treatments only, were available to act as a buffer against the conditions that caused the soils in Clipped Treatments to become more acidic (Shi, et al., 2011).

The extracellular enzyme phenol oxidase belongs to the oxidoreductase enzyme group utilizes oxygen as an electron acceptor to oxidize phenolic compounds (Reddy and DeLaune, 2008). Plants and microbes produce extracellular phenol oxidase to gain access to recalcitrant forms of carbon in SOM such as lignin and tannins. Subsequently, microbes can absorb these carbon compounds for metabolism; as a result of metabolism, CO₂ is generated and taken up by plant roots for primary productivity (Sinsabaugh, 2010). Optimal pH levels for this substrate vary widely, however most favorable conditions are commonly acidic (Sinsabaugh, 2010, Snajrd et al., 2008). Unlike hydrolytic enzymes, phenol oxidase activity is highly sensitive to the availability of oxygen (Reddy and DeLaune, 2008) and redox potentials for high EEA range

from 450 to 800 mV (Sinsabaugh, 2010). In this study, EEA for phenol oxidase was significantly higher at the Dry Site than the Intermediate or Wet sites (Figure 8, Table 5). Higher EEA observed at the Dry Site is likely a result of aerobic conditions and acidic pH levels, which are confirmed by the RDA biplots (Figure 16; A, B) and the Spearman rank correlation matrix (Table 4). At the Dry Site, Vegetated Treatments exhibited lower EEA rates than Clipped Treatments, which is likely due to the availability of simple labile carbons produced by above-ground biomass that are less abundant in soils lacking vegetation. The presence of simple carbon compounds negated the incentive for microbes to breakdown recalcitrant carbons for a source of energy. This was evidenced by a strong negative correlation between below-ground root biomass and EEA, regardless of treatment (Figure 16; A, B). Also due to the presence of simple carbon compounds, no correlation was found between EEA and C:N in Vegetated Treatments; alternatively, a strong correlation was found in Clipped Treatments. The lack of new OM inputs from plant detritus during the duration of the study caused the C:N ratio in the soils of Clipped Treatments to increase, and as a result, a decline in SOM quality lead to lower EEA.

2.2. *S, P, and N Substrates*

Plants uptake sulfur in the form of inorganic sulfates (SO_4^{-2}), which are fixed into amino acids or proteins. Once this happens, these compounds must undergo mineralization and mobilization by extracellular enzymes to become biologically re-available. Arlylsulfatase hydrolyzes unavailable sulfate esters or oxidizes soluble OM in response to sulfur limited environments (Makoi and Ndakidemi, 2008). The rate at which sulfate is made biologically available is dependent on pH, SOM content and quality, and soil saturation (Makoi and Ndakidemi, 2008; Xiangzhen and Pariente, 2003). Results from this study confirm some of

these claims as witnessed in strong positive correlations of arylsulfatase activity to both saturation and pH, which explained much of the variance across sites (Figure 7). This was only true in Vegetated Treatments (Figure 16; A, B). In Vegetated Treatments, SOM and above-ground plant biomass were also strongly correlated to EEA (Figure 16, A) due to a significantly larger accumulation of high quality SOM in the Vegetated Treatments than Clipped Treatments. Also contributing to differences across sites was plant community composition; evidence of this relationship was obtained from a strong Mantel correlation between arylsulfatase activity and plant community composition (Table 7). As in the case of other plant limiting nutrients, arylsulfatase rates are highest in the Vegetated Treatments. It is within this environment where a plant-microbe nutrient competition increases rates of sulfur reduction and oxidation. Similar results were found in a study by Xiangzhen and Pariente (2003), who observed significantly higher arylsulfatase rates in the presence of vegetation (denoted as “under shrub”) compared to sites where vegetation was absent (denoted as “between shrub” and “under rock”).

Alkaline phosphatase is a hydrolytic enzyme that plays a key role in regulating the hydrolysis of organic phosphates into inorganic orthophosphates that are biologically available (Reddy and DeLaune, 2008; Mubyana et al., 1998). Higher alkaline phosphatase activity observed in Vegetated Treatments indicates a plant-microbe relationship under phosphorous limited conditions (Makoi and Ndakidemi, 2008; Reddy and DeLaune, 2008). The bioavailability of organic phosphorous is regulated by phosphorous mineralization; therefore, the nutrient demands of above-ground biomass caused an increased rate in activity, and conversely, activity decreased when nutrient demand was low (Reddy and DeLaune, 2008; Xiangzhen and Pariente, 2003). Evidence of this relationship is found in Table 4, revealing significant positive correlations between EEA and both above and below-ground biomass. Results showing higher EEA activity

under anaerobic conditions are confirmed by a study by Mubyana et al. (1998), who observed alkaline phosphatase rates to be lowest in dry soil and continuously higher as soil saturation increased. Inundation increases alkaline phosphatase activity by increasing the solubility of cations such as iron and aluminum, which control the solubility and availability of phosphorous in the soil profile (Mubyana et al., 1998). Activity was also lower at the Dry Site than the Intermediate or Wet sites because acidic soils like those found at the Dry Site (Table 3) limit the rate of synthesis, release, and stability of alkaline phosphatase (Eivazi and Tabatabai, 1977; Juma and Tabatabai, 1977; Tabatabai, 1994; Martinez and Tabatabai, 2000).

Leucyl aminopeptidases (LAPs) cleave N-terminal residues from proteins and peptides (Matsui, et al., 2006). LAPs are known as cell maintenance and defense enzymes with critical roles in the turnover of peptides; in addition, LAPs assure complete recycling of amino acids from peptides created by endoproteases (Reddy and DeLaune, 2008). LAPs have variable temperature, pH, and divalent cation requirements (Matsui, et al., 2006). In this study LAP activity was highest at the Wet Site and lowest at the Dry Site; furthermore, activity was higher in Vegetated Treatments than Clipped Treatments. These findings are consistent with claims by Matsui et al. (2006) that pH is a significant explanatory variable for changes in LAP activity. The RDA biplot (Figure 16; A, B) demonstrated a close positive correlation with soil pH regardless of treatment. Below-ground biomass was positively correlated with EEA in Vegetated Treatments only; likewise, only in the absence of vegetation did SOM strongly influence EEA (Figure 16; A, B). LAP activity in Vegetated Treatments was positively affected by root exudates that were absent in Clipped Treatments. Without exudates providing substrate to the LAPs they became reliant on available nitrogen substrate in the SOM. In addition, a strong positive relationship was observed with soil temperature ($r_s = 0.51$, Table 4) and, unlike

the aforementioned extracellular enzymes, leucyl aminopeptidase rates were not consistent across all months of the study period (Table 6).

In addition to the relationship of LAP with environmental properties, there appears to be a strong effect of vegetation on leucyl aminopeptidase activity. A significant site x treatment interaction effect was found in May, July, and September; this effect disappeared in November during plant senescence. This indicates that the effect of site is contingent on the presence of living plant biomass. Further, the Spearman rank correlation matrix found LAP activity to be strongly positively correlated to above-ground plant biomass ($r_s = 0.43$), indicating that root exudates are a major source of nitrogen that can be readily broken down by LAPs. However, the type of vegetation present did not seem to be an important factor ($r_m = -0.04$, $p = 0.5094$). As with other extracellular enzymes in this study, activity rates increased when plants and microbes were in competition for nutrients (i.e., the “Vegetated Treatment”), in this case, biologically available nitrogen. Rates were highest at the Wet Site due to more alkaline pH, greater plant biomass, and more stable soil temperatures maintained by increased inundation (Matsui et al., 2006). Rates may also have been higher due to a greater availability of nitrogen for assimilation, which could have been present as a result of greater nitrogen accumulations in the SOM at the Wet Site than drier sites (Reddy and DeLaune, 2008; Matsui, et al., 2006). The net effect of mineralization and immobilization is higher under flooded conditions, which leads to higher rates of inorganic nitrogen accumulation (Reddy and DeLaune, 2008). The bioavailability of nitrogen in the soil at the Wet Site is likely lower than other sites due to reduced anaerobic conditions; subsequently, the influence of root exudates and the rate of EEA increase as the plant and microbial communities become strained.

Conclusions

This research demonstrated a significant and interactive effect of soil moisture and vegetation on the function of microbial communities in wetland soils. For most enzymes, a site difference was observed due to soil moisture content, which had an effect on soil pH, redox potential, and plant community composition. All hydrolytic extracellular enzymes responded favorably to wetter environments; only the oxidoreductase extracellular enzyme showed greater activity in dryer sites. For most enzymes, the presence of vegetation increased activity. The effect of vegetation on EEA was caused by species composition, root biomass, and/or above-ground plant biomass, depending on the extracellular enzyme in question. Based on different soil characteristics at the Wet, Intermediate, and Dry sites, as well as differences between Vegetated and Clipped treatments, saturation and plant presence appear to be key factors shaping soil physicochemical properties, and subsequently regulating EEA. The presence of a vegetation effect in year two of this study suggests that microbial community function does not immediately change following environmental disturbances, however if environmental changes are maintained the effect will continually increase over time. It is also important to note that when undertaking wetland restoration projects reestablishing hydrologic and vegetated conditions are paramount in achieving previous functionality. Further research should investigate the role of specific plant species compositions as well as soil pore water chemistry to better understand variability across sites and treatments.

Literature Cited

Literature Cited

- Bever, J D., Westover, K. M., & Antonovics, J. (1997). Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *Journal of Ecology*, 85, 561-573.
- Blankenship, Karl (1994). 'No net loss' proves to be an elusive goal. *Chesapeake Bay Journal*, 4(2).
- Bossio, Deborah A., Fleck, Jacob A., Scow, Kate M., Fujii, Roger (2006). Alteration of soil microbial communities and water quality in restored wetlands. *Soil Biology & Biochemistry*, 38, 1223-1233.
- Card, S.M., Quideau, S.A. (2010). Microbial community structure in restored riparian soils of the Canadian prairie pothole region. *Soil Biology & Biochemistry*, 42, 1463-1471.
- Cowardin, L.M., Carter, V., Golet, F.C. and La Roe, E.T. 1979. Classification of wetlands and deepwater habitats in the United States. U.S. Dept. Interior, Fish & Wildlife Service, FWS/OBS-79/31.
- Eivazi F, Tabatabai MA (1977). Phosphates in soils. *Soil Biol. Biochem.* 9: 167-172.
- Geisseler, Daniel and Horwath, William R. (2009) Relationship between carbon and nitrogen availability and extracellular enzyme activities in soil. *Pedobiologia*, 53(1), 87-98.
- Gower, J.C. (1971). A general coefficient of similarity and some of its properties. *Biometrics*, 27, 857-876.
- Gutknecht, Jessica L. M., Goodman, Robert M., Balser, Teri C. (2006). Linking soil process and microbial ecology in freshwater wetland ecosystems. *Plant Soil*, 289, 17-34
Environment. *Soil Science Society of America, American Society of Agriculture*, Madison, pp. 107-124.
- Hammer, O., Harper, D., & Ryan, P. D. (2005). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4(1), 9.
- Jenkins, A.S. and R.B. Franklin. In review. Depth and time related variations of microbial communities in an emergent freshwater wetland. *Wetlands*.
- Juma NG, Tabatabai MA (1977). Effects of trace elements on phosphatase activity in soils. *SSSAJ* 41, 343-346.

- Legendre, Pierre and Anderson, Marti J. (1999). Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. *Ecological Monographs*, 69(1), 1-24.
- Legendre, Pierre & Louis Legendre. 1998. Numerical ecology, 2nd English edition: Developments in Environmental Modeling 20. Elsevier Science BV, Amsterdam. xv + 853 pages.
- Makoi, Joachim, H.R. and Ndakidemi, Patrick, A. (2008). Selected soil enzymes: Examples of their potential roles in the ecosystem. *African Journal of Biotechnology*. 7(3), 181-191.
- Martinez CE, Tabatabai MA (1997). Decomposition of biotechnology by-products in soils. *J. Environ. Qual.* 26: 625-632.
- Marx, M.C., Wood M., Jarvis, S.C. (2001). A microplate fluorimetric assay for the study of enzyme diversity in soils. *Soil Biology & Biochemistry*, 33, 1633-1640.
- Matsui, M., Fowler, J. H., and Walling, L. L. (2006) Leucine aminopeptidases: diversity in structure and function. *Biol. Chem.* 387, 1535-1544.
- Mitsch, William J., Gosselink, James G. (2000). Wetlands: Third Edition (3rd ed.) New York, NY: John Wiley and Sons.
- Mentzer, Jessica L., Goodman, Robert M., Balser, Teri C. (2006). Microbial response over time to hydrologic and fertilization treatments in a simulated wet prairie. *Plant and Soil*, 284, 85-100.
- Mubyana, Tabo, Krah, Martin, Totolo, Otlogetswe, and Bonyongo, Mpaphi (2003). Influence of seasonal flooding on soil total nitrogen, organic phosphorous and microbial populations in the Okavango Delta, Botswana. *Journal of Arid Environments*, 54(2), 359-369.
- Peres-Neto, Pedro R., Legendre, Pierre, Dray, Stephane, and Borcard, Daniel (2006). Variation partitioning of species data matrices: estimation and comparison of fractions. *Ecology*, 87(10), 2614-2625.
- Prasse, Christine (2010). Hydrology and vegetation effects on microbial community structure and extracellular enzyme activity in the sediments of freshwater wetlands. Unpublished.
- Rao, C. R. (1964). The use and interpretation of principal component analysis in applied research. *Sankhyaa*, Series A 26, 329-358.
- Reddy, Ramesh K. and DeLaune, Ronald D. (2008) Biogeochemistry of Wetlands: Science and Applications. CRC Press, New York, NY.

- Sinsabaugh, R. L., Linkins, A. E., & Antibus, R. K. (1991). An enzymatic approach to the analysis of microbial activity during plant litter decomposition. *Agriculture, Ecosystems and Environment*, 34, 43-54.
- Sinsabaugh R.L., Hill B.H., Follstad Shah J.J. (2009). Eoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. *Nature*, in press.
- Sinsabaugh, R. L. (2010). Phenol Oxidase, peroxidase, and organic matter dynamics of soil. *Soil Biology and Biochemistry*. 42(3), 391-404.
- Shi, Shengjing, Richardson, Allen E., O'Callaghan, Maureen, DeAngelis, Kristen M., Jones, Eirian E., Stewart, Alison, Firestone, Mary K., Condon, Leo M. (2011). Effects of selected root exudate components on soil bacteria communities. *FEMS Microbiology Ecology*. doi: 10.1111/j.1574-6941.2011.01150.x
- Steinweg, M., McMahon, (2007). Downloaded from Ecosystem & Soil Ecology Laboratory, University of Toledo.
<<http://www.eeescience.utoledo.edu/faculty/weintraub/ESELab.htm>>
- Tabatabai MA (1994). Soil enzymes. In: Weaver RW, Angle JS, Bottomley PS (eds) Methods of soil analysis, part 2. Microbiological and biochemical properties. SSSA Book Series No. 5. *Soil Sci. Soc. Am.* Madison, Wis., pp. 775-833.
- Thomas, Cassondra R., Miao, ShiLi, Sindhoj, Erik (2009). Environmental factors affecting temporal and spatial patterns of soil redox potential in Florida everglades wetlands. *Wetlands*, 29(4), 1133-1145.
- Wardle, David A., Bardgett, Richard D., Klironomos, John N., Setälä, Heikki, van der Putten, Wim H., Wall, Diana H. (2004). Ecological linkages between aboveground and belowground biota. *Science*, 304, 1629-1633.
- Westover, Kristi M., Kennedy, Ann C., Kelley, Steven E. (1997). Patterns of rhizosphere microbial community structure associated with co-occurring plant species. *Journal of Ecology*, 85, 863-873.
- Wiessner, A., Kuschik, P., Jechorek, M., Seidel, H., Kastner, M. (2007). Sulphur transformation and deposition in the rhizosphere *Juncus effusus* in a laboratory-scale constructed wetland. *Environmental Pollution*, 155, 125-131.
- Windham-Myers, Lisamarie, Marvin-Dipasquale, Mark, Krabbenhoft, David P., Agee, Jennifer L., Cox, Marisa H., Heredia-Middleton, Pilar, Coates, Carolyn, and Kakouros, Evangelos (2009). Experimental removal of wetland emergent vegetation leads to decreased methylmercury production in surface sediment. *Journal of Geophysical Research*, 114, G00C05, 14 PP.

- Xiangzhen, Li and Pariente, Sarah (2003). Arylsulfatase activity of soil microbial biomass along a Mediterranean-arid transect. *Soil Biology and Biochemistry*, 35(7), 925-934.
- Zak, Donald R., Holmes, William E., White, David C., Peacock, Aaron D., Tilman, David (2003). Plant diversity, soil microbial communities, and ecosystem function: are there any links? *Ecology*, 84(8), 2042-2050

Appendix

Table 1: Summary of substrates, solvents, concentrations, and reaction times used to assess soil extracellular enzyme activity.

Enzyme	EC#	Substrate	Sigma-Aldrich Catalog Number	Solvent	Assay Concentration (mM)	Total Reaction Time (H)
β -1,4-glucosidase	3.2.1.21	4-MUB β -D-glucopyranoside	M3633	EGME* *	0.60	6.5
1,4- β –cellobiosidase	3.2.1.91	4-MUB β -D-cellobioside	M6018	EGME	0.60	1.25
β -D-xylosidase	3.2.1.37	4-MUB- β -D-xylopyranoside	M7008	EGME	0.60	6.0
Phenol Oxidase	1.10.3.2	L-DOPA*	D9629	Sterile DI H ₂ O	6.25	4.5
Leucyl aminopeptidase	3.4.11.1	L-Leucine-7-amido-4-methylcoumarin HCl	L2145	Sterile DI H ₂ O	0.40	2.25
Arylsulfatase	3.1.6.1	4-MUB-sulfate	M7133	EGME	0.60	5.25

* L-DOPA - 3,4-Dihydroxy-L-phenylalanine

** EGME – ethylene glycol monomethyl ether (methylcellosolve) is used to stabilize the substrate

Table 2: Plant species richness of each site throughout the study. “X” indicates the presence of living biomass for a particular plant type.

		May			July			September			November		
Scientific Name	Common Name	Dry	Interm	Wet	Dry	Interm	Wet	Dry	Interm	Wet	Dry	Interm	Wet
Common to Multiple Sites													
Agrostis sp.	Bentgrass	X	X		X	X			X	X		X	X
Boehmeria cylindrica	False Nettle	X			X		X	X	X		X	X	
Commelina communis	Asiatic Dayflower				X	X			X				
Hibiscus moschentos	Marsh Hibiscus	X	X		X	X		X	X		X		
Juncus effusus	Soft Rush	X	X	X	X	X	X		X	X	X	X	X
Leersia oryzoides	Rice Cut Grass		X	X	X	X	X		X	X	X	X	X
Ludwigia palustris	Water Purslane			X		X	X			X			X
Murdannia keisak	Asian Spiderwort				X			X	X	X	X		
Polygonum safitatum	Sagitate Tearthumb		X		X	X	X		X		X		X
Saccharum alopecuroides	Silver Plume Grass				X	X		X	X		X	X	
Exclusive To A Given Site													
Acer rubrum	Red Maple	X			X								
Acorus calamus	Sweet Flag				X								
Alnus serrulata	Smooth Alder				X						X		
Asteraceae family	Unidentified Aster	X			X			X			X		
Carex lurida	Sallow Sedge	X											
Cladium jamaicense	Sawgrass					X							
Clethra alnifolia	Sweet Pepper Bush				X								
Diospyros digyna	Persimmon				X			X					
Eupatorium dubium	Joe-Pye Weed							X			X		
Echninochloa walteri	Walter's Millet							X					
Fraxinus pennsylvanica	Green Ash		X										
Hypericum mutilum	Dwarf Staint John's Wart				X			X					
Liquidambar stycaciflua	Sweet Gum				X			X			X		
Nyssa biflora	Black Gum	X			X			X					
Phytolacca americana	American Pokeweed				X								
Polygonum cespitosum	Oriental Lady's Thumb							X					
Polygonum punctatum	Dotted Smartweed	X			X								
Sagittaria latifolia	Arrowhead Duck Potato									X			X
Setaria parviflora	Foxtail				X			X			X		
Scirpus cyperinus	Woolgrass									X			
Typha angustifolia	Narrow-Leaved Cattail			X			X			X			X

Table 3: Two-way ANOVA for all soil and vegetation properties with a site x treatment interaction.

Soil Characteristic	Site x Treatment Interaction		Main effect: Site		Site means (+/- SE)			Main effect: Treatment		Treatment means (+/- SE)	
	F	p	F	p	Wet	Intermediate	Dry	F	p	Veg	Clipped
Soil Saturation (%)	1.54	0.2198	445.80	< 0.001*	176.1 (4.2)	105.3 (2.9)	43.5 (2.4)	10.68	0.0014*	114.2 (7.8)	102.4 (7.2)
pH	0.93	0.3969	95.45	< 0.001*	5.7 (0.1)	5.3 (0.1)	3.8 (0.1)	0.67	0.4148	4.9 (0.1)	5.0 (0.1)
Redox (mV)	1.07	0.3481	344.80	< 0.001*	-36.2 (10.4)	27.5 (21.3)	459.0 (8.1)	0.00	0.9727	149.8 (30.2)	150.4 (31.7)
Soil Organic Matter (%)	4.21	0.0172*	N/A	N/A	10.6 (7.9)	7.9 (0.3)	9.7 (1.4)	N/A	N/A	8.7 (0.3)	102 (1.0)
C:N	5.08	0.0077*	N/A	N/A	12.1 (0.2)	12.2 (0.2)	13.9 (0.3)	N/A	N/A	12.6 (0.2)	12.9 (0.2)
Roots (g/cm ³)	0.73	0.4845	2.73	0.0694	0.011 (0.002)	0.009 (0.001)	0.007 (0.001)	38.22	< 0.001*	0.014 (0.001)	0.004 (0.001)
Soil Temperature (C°)	0.23	0.7982	1.16	0.3177	18.3 (0.7)	18.8 (0.8)	19.9 (0.8)	0.01	0.9165	19.0 (0.6)	18.9 (0.6)

* significance based on $\alpha = 0.05$

Table 4: Spearman Rank Correlation (r_s) between environmental variables and enzyme rates.

	Saturation	pH	Redox	SOM	BG-B	C:N	AG-B	Temp	BG	Cell	Xylo	PO	Sulfatase	Phosphate	LAP
Saturation	0														
pH	0.69*	0													
Redox	-0.72*	-0.90*	0												
SOM	0.52*	0.25*	-0.21*	0											
BG-B	0.22**	-0.05	0.01	0.28*	0										
C:N	-0.51*	-0.48*	0.50*	-0.21*	-0.03	0									
AG-B	0.20*	0.06	-0.09	0.16	0.59*	-0.08	0								
Temp	-0.21*	-0.18*	0.25*	-0.11	-0.08	0.21	-0.16	0							
BG	0.58*	0.43*	-0.46*	0.35*	0.34*	-0.36*	0.61*	-0.53*	0						
Cell	0.84*	0.52*	-0.58*	0.45*	0.23*	-0.44*	0.04	0.03	0.35*	0					
Xylo	0.31*	-0.01	-0.13	0.18	0.42*	0.16	0.21	0.25*	0.04	0.43*	0				
PO	-0.43*	-0.31*	0.33*	-0.10	-0.29*	0.27*	-0.23*	0.23*	-0.38*	-0.47*	-0.25*	0			
Sulfatase	0.56*	0.43*	-0.37*	0.34*	0.16	-0.42*	0.32*	-0.18	0.57*	0.51*	-0.16	-0.31*	0		
Phosphate	0.48*	0.16	-0.23*	0.31*	0.42*	-0.23*	0.52*	-0.59*	0.75*	0.29*	0.22	-0.37*	0.41*	0	
LAP	0.42*	0.27*	-0.24*	0.27*	0.27*	-0.13	0.43*	0.51*	0.27*	0.46*	0.29*	-0.14	0.47*	0.01	0

Significance based on $p < 0.003$ (Bonferroni corrected for multiple comparisons)

β -1,4 glucosidase = BG

1,4- β – cellobiosidase = Cell

β -D-xylosidase = Xylo

Alkaline phosphatase = Phosphate

Arlylsulfatase = Sulfatase

Leucyl Aminopeptidase = LAP

Phenol oxidase = PO

Temperature = Temp

Above-ground plant biomass = AG-B

Below-ground root biomass = BG-B

Table 5: Two-way ANOVA for all extracellular enzyme rates with a site x treatment interaction.

Extracellular Enzyme	Site x Treatment Interaction		Main effect: Site		Site means (+/- SE)			Main effect: Treatment		Treatment means (+/- SE)	
	F	p	F	p	Wet	Intermediate	Dry	F	p	Vegetated	Clipped
β -D-xylosidase	0.08	0.9221	7.42	0.001*	0.498 (0.041)	0.358 (0.034)	0.316 (0.031)	12.24	< 0.001*	0.461 (0.034)	0.320 (0.026)
Phenol Oxidase	4.48	0.0134*	N/A	N/A	1.297 (0.254)	1.369 (0.178)	3.108 (0.324)	N/A	N/A	1.488 (0.185)	2.358 (0.268)
β -1, 4 glucosidase	1.27	0.2851	24.17	< 0.001*	0.271 (0.021)	0.179 (0.017)	0.131 (0.016)	59.66	< 0.001*	0.258 (0.016)	0.129 (0.012)
Alkaline Phosphatase	0.23	0.7921	11.67	< 0.001*	3.442 (0.174)	2.590 (0.169)	2.433 (0.180)	26.25	< 0.001*	3.292 (0.153)	2.351 (0.126)
Arlylsulfatase	1.73	0.1828	25.60	< 0.001*	0.254 (0.019)	0.195 (0.016)	0.121 (0.009)	24.66	< 0.001*	0.228 (0.015)	0.152 (0.012)
1,4- β – cellobiosidase	1.18	0.3120	98.94	< 0.001*	0.941 (0.036)	0.691 (0.024)	0.434 (0.016)	0.80	0.3741	0.692 (0.032)	0.665 (0.032)
Leucyl Aminopeptidase	See Table 6										

* significance based on $\alpha = 0.05$

EEA rates = $\mu\text{mol activity g dry-1 soil H-1}$

Table 6: ANOVA results for Leucyl Aminopeptidase and mean (\pm SE) for each individual treatment x site combination

Month	Overall Site x Treatment		Mean (\pm SE)					
	F	P	Wet- Vegetated	Wet- Clipped	Intermediate- Vegetated	Intermediate- Clipped	Dry- Vegetated	Dry- Clipped
May	18.67	< 0.001*	0.3957 (0.0248)	0.1812 (0.0087)	0.2873 (0.0169)	0.1254 (0.0139)	0.2047 (0.0106)	0.1719 (0.0111)
July	3.58	< 0.001*	0.8260 (0.0659)	0.6560 (0.0330)	0.5309 (0.0252)	0.4044 (0.0232)	0.3012 (0.0023)	0.3054 (0.0197)
September	12.53	< 0.001*	0.4434 (0.0098)	0.1312 (0.0410)	0.3509 (0.0138)	0.0932 (0.0144)	0.1825 (0.0060)	0.0584 (0.0058)
November	0.41	0.67	0.2426 (0.0212)	0.1801 (0.0108)	0.1842 (0.0167)	0.1269 (0.0119)	0.1539 (0.0130)	0.1153 (0.0019)

*significance based on $\alpha = 0.05$

EEA rates = $\mu\text{mol activity g dry}^{-1} \text{ soil H}^{-1}$

Table 7: Mantel correlations (r_m) for both EEA rates and soil variables to explore the relationship with plant species composition

Mantel Correlations (r_m)	
	Plant Species Composition
Extracellular Enzyme Activity	
1,4- β – cellobiosidase	0.57*
β -1,4 glucosidase	0.35*
Arylsulfatase	0.54*
Alkaline phosphatase	-0.01
Leucyl aminopeptidase	-0.04
β -D-xylosidase	-0.08
Phenol oxidase	0.05
Environmental Soil Properties	
All Environmental Variables	0.44*
Soil Moisture Content	0.78*
Soil Organic Matter	0.46*
pH	0.47*
Redox	0.65*
C:N	0.21
Below-ground root biomass	0.04
Above-ground plant biomass	-0.1
Temperature	-0.22

* Significance based on $\alpha = 0.05$

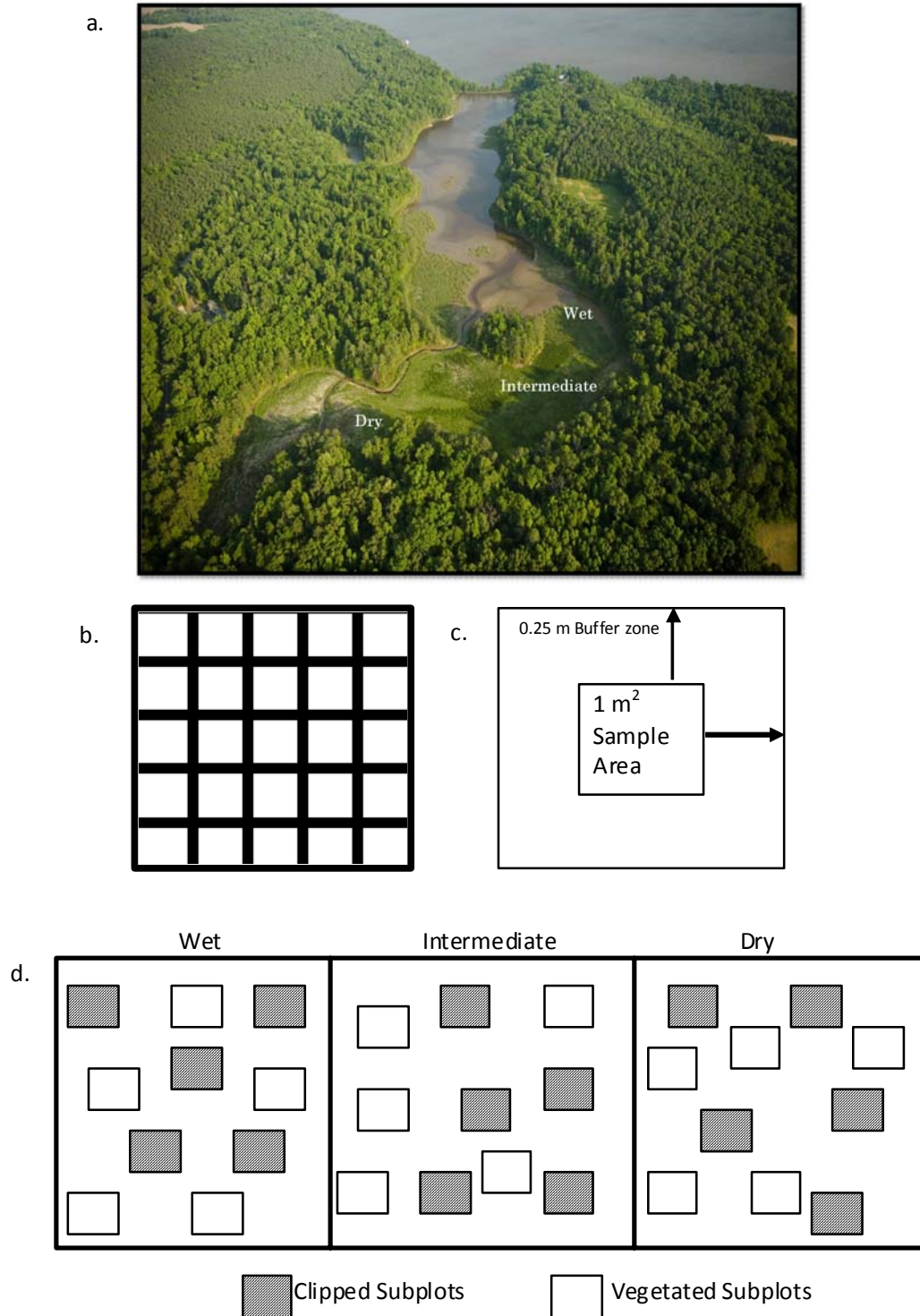


Figure 1: Experimental Design: (a) Research site labeled with each main plot location (b) Each main 7.5 m x 7.5 m plot was established in three different locations (not adjacent) then subdivided into (25) 1.25 m x 1.25 m subplot; (c) Five non-vegetated and reference subplots were randomly selected from the grid system and sampled every eight weeks (the above diagram does not reflect actual plot location). (d) Samples were collected from the internal 1.0 m² zone to minimize edge effect.

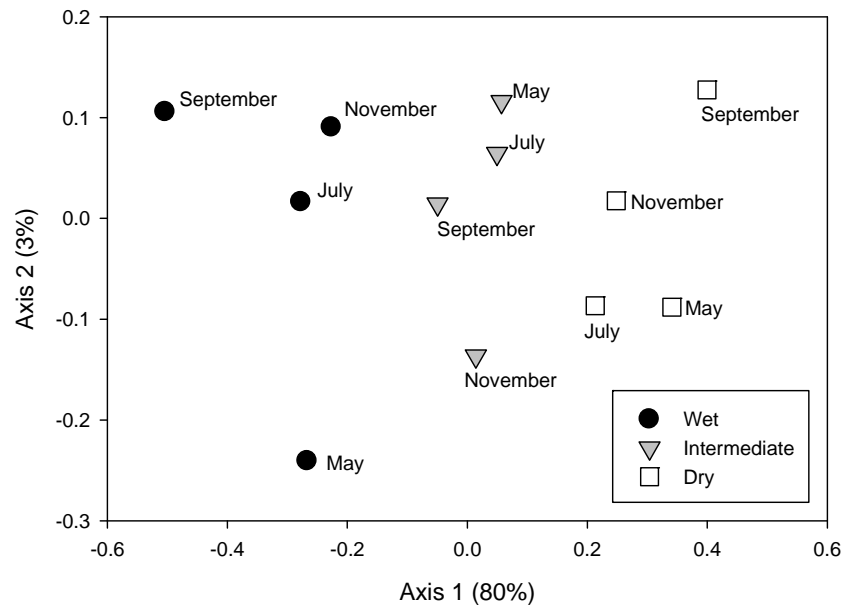


Figure 2: NDMS using Sorensen Index to analyze plant community composition (Stress = 0.12). Plotted points are based on binary data (presence/absence).

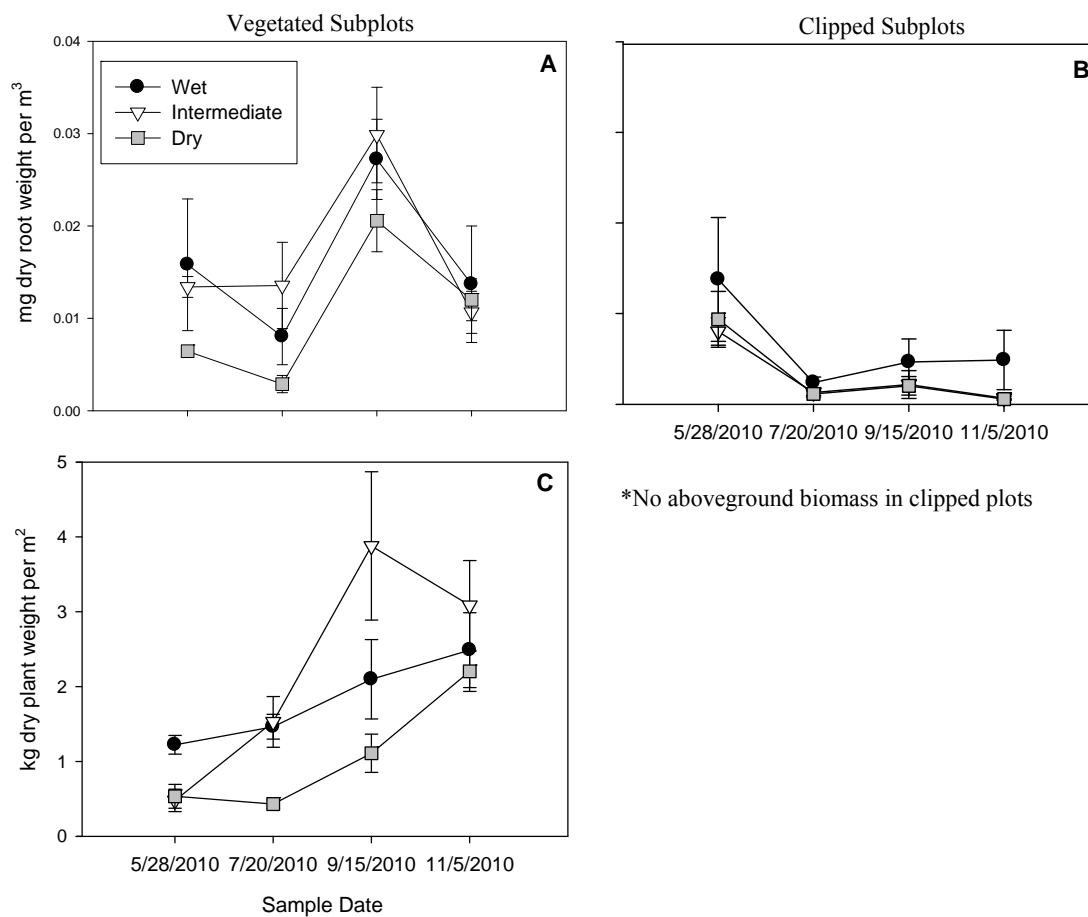


Figure 3: Vegetated and clipped subplot mean \pm standard error for belowground root biomass (A)(B), aboveground plant biomass (C) for each month assessed.

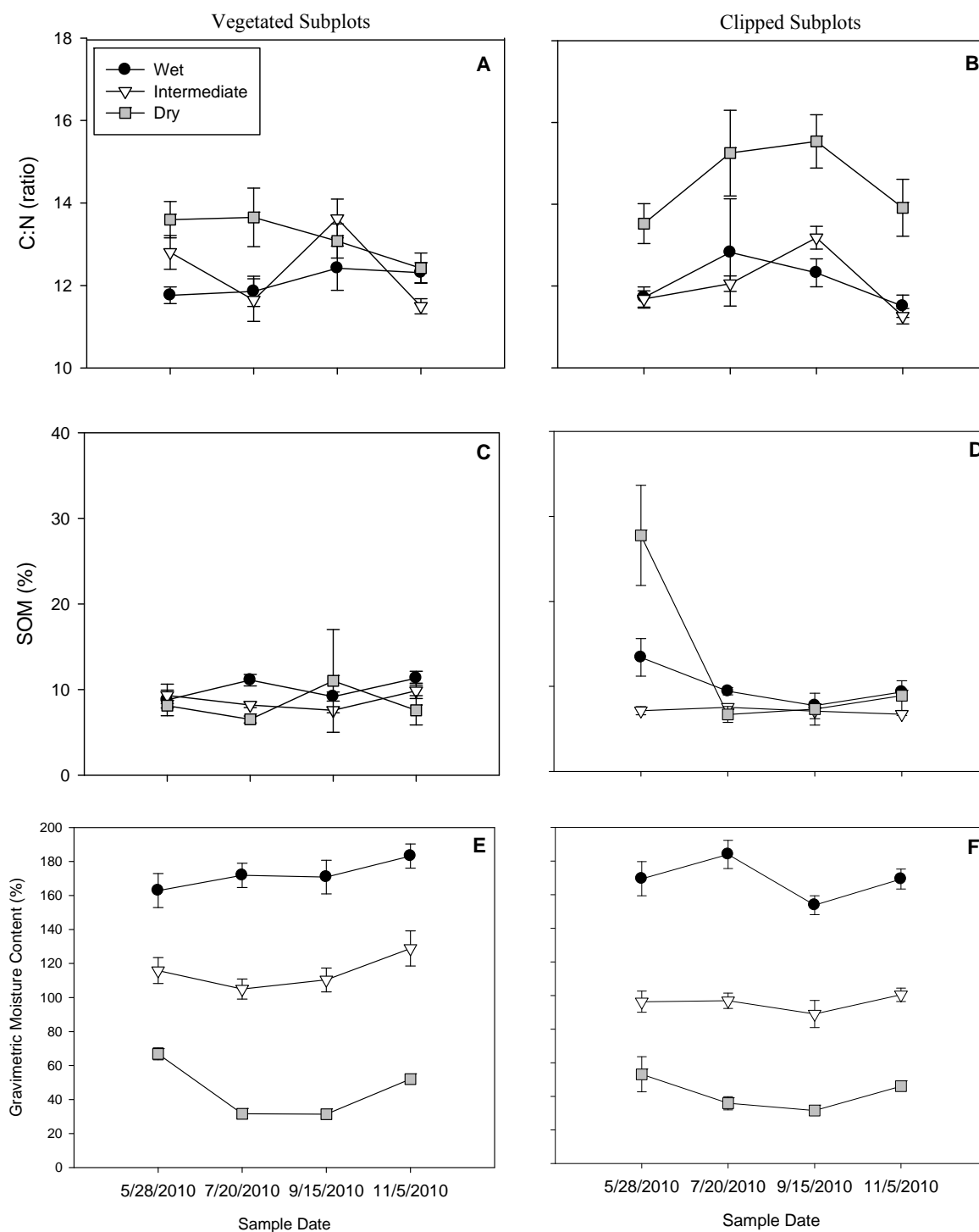


Figure 4: Vegetated and clipped subplot mean \pm standard error for C:N ratio (A)(B), soil organic matter (C)(D), and soil moisture (E)(F) for each month assessed.

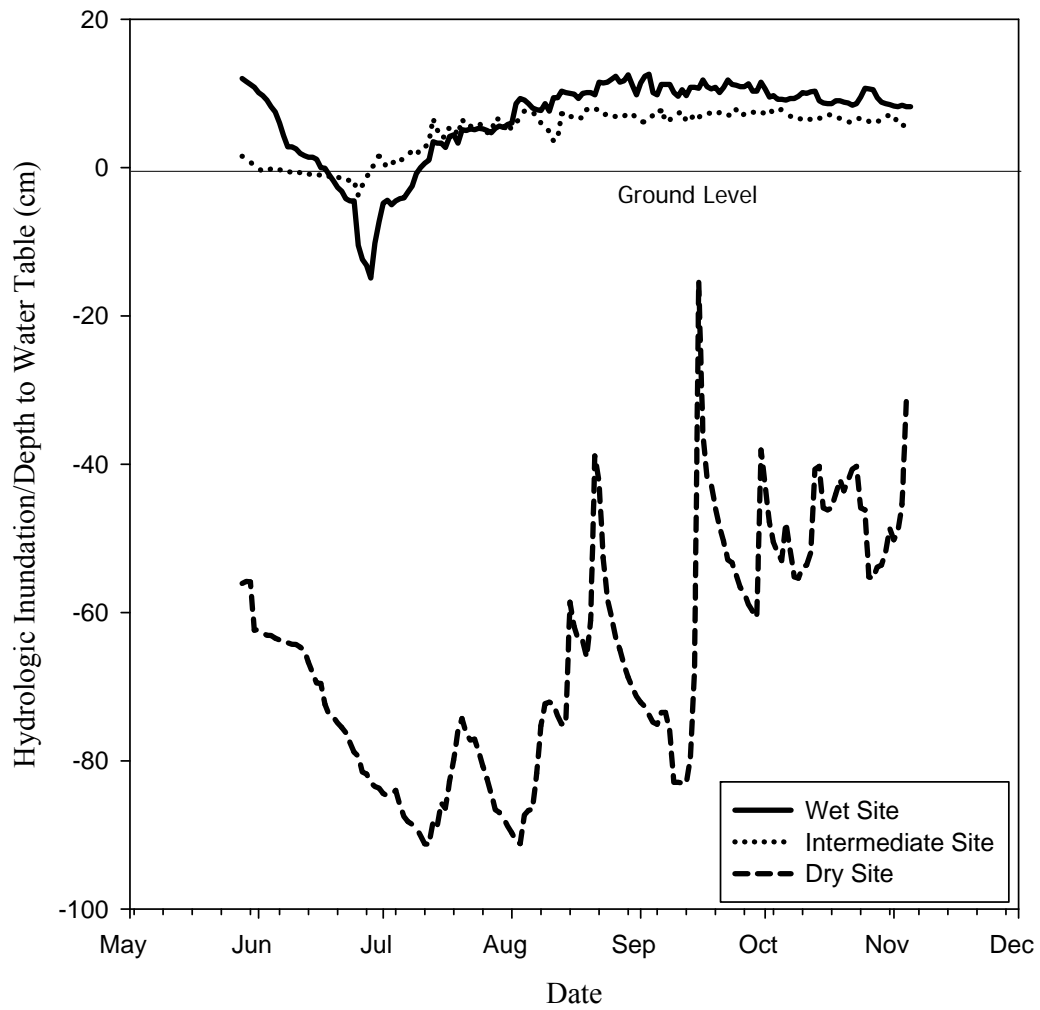


Figure 5: Groundwater level over the entire length of the study. One measurement per day is plotted for each site.

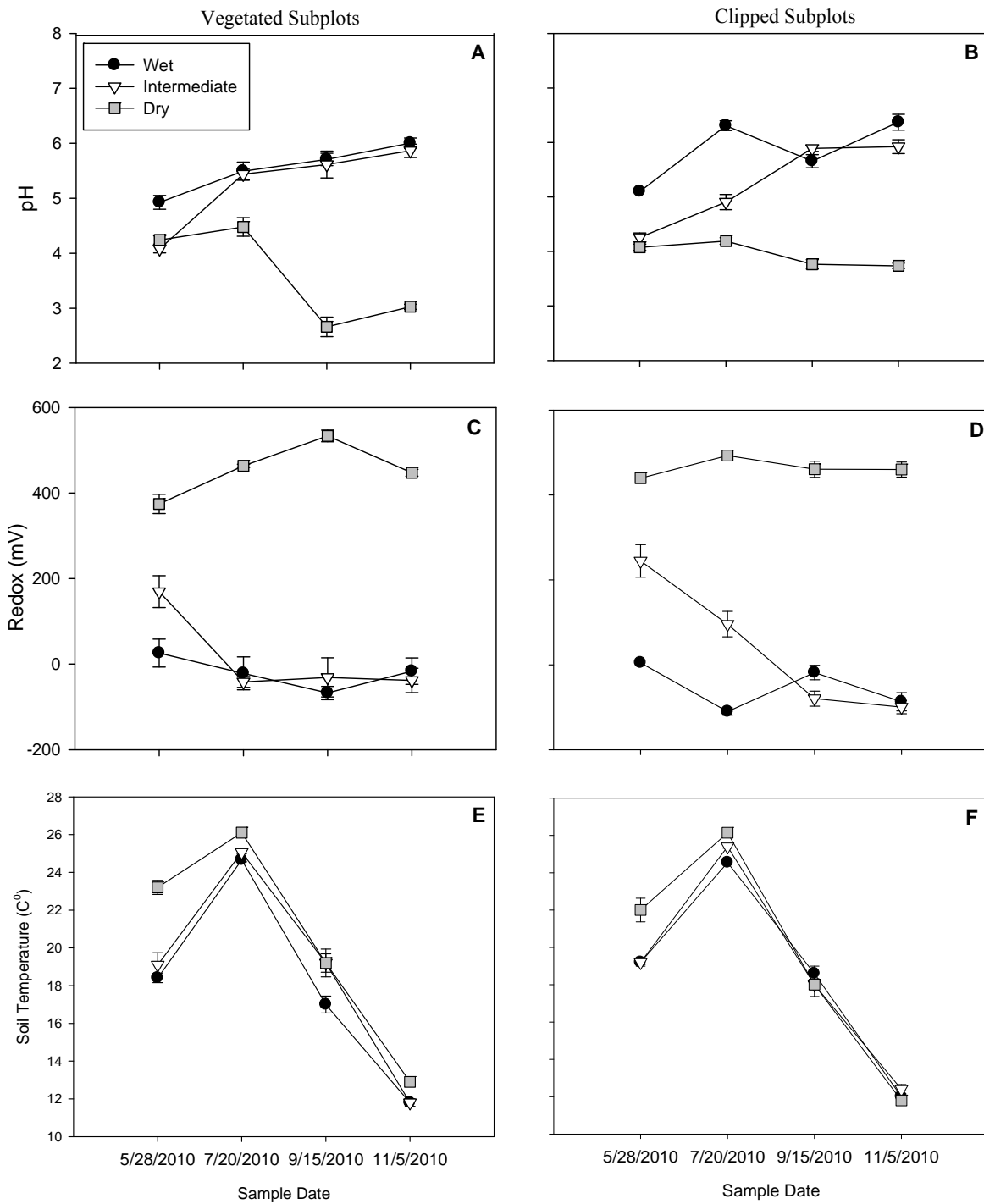
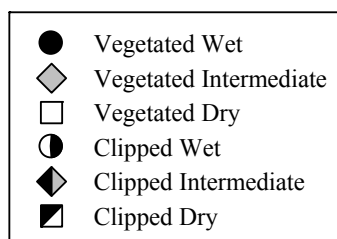
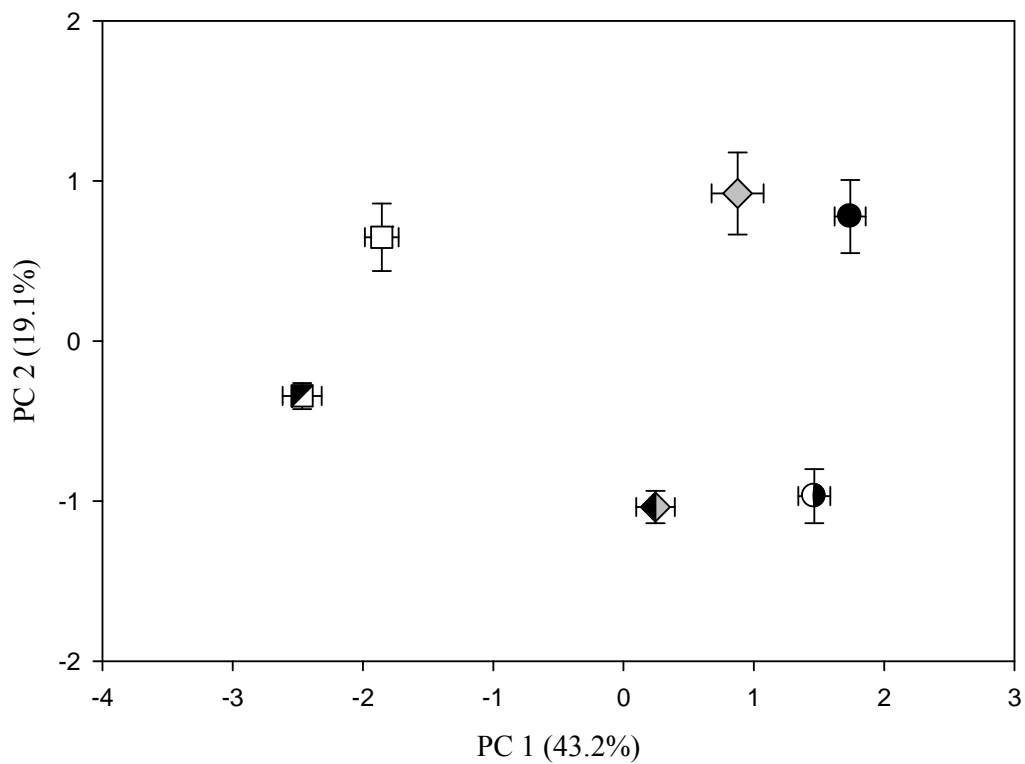


Figure 6: Vegetated and clipped subplot mean \pm standard error for pH (A)(B), redox (C)(D), and soil temperature (E)(F) for each month assessed.

Figure 7: PCA of all environmental properties excluding temperature. Individual points are centroids (N = 20) with all sampling events, and error bars \pm SE.



Factor Loadings
PC 1 :
Redox (-0.94)
pH (0.89)
Soil Saturation (0.88)

PC 2 :
Roots (0.84)
Plants (0.74)

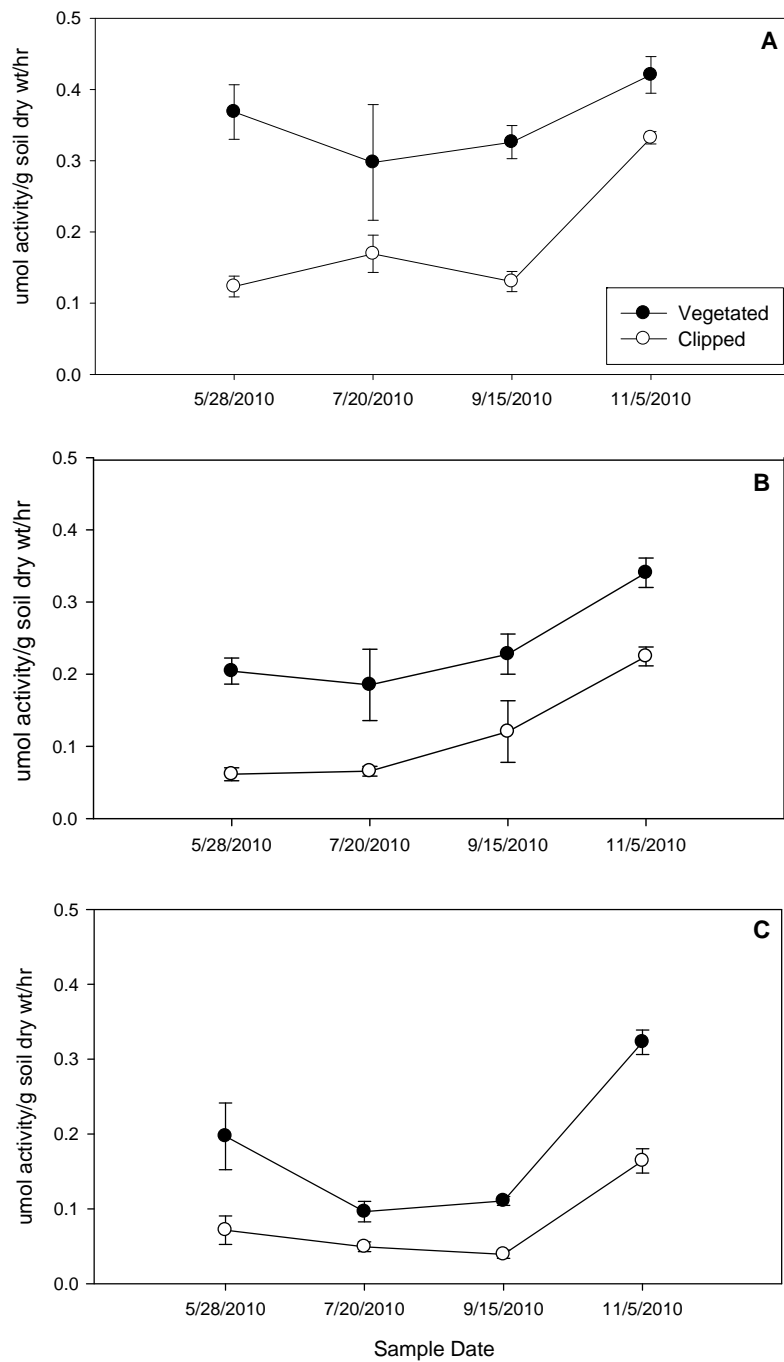


Figure 8: Mean \pm standard error for extracellular enzyme activity of β -1,4-glucosidase in the (A) Wet Site, (B) Intermediate Site, and (C) Dry Site for each month assessed.

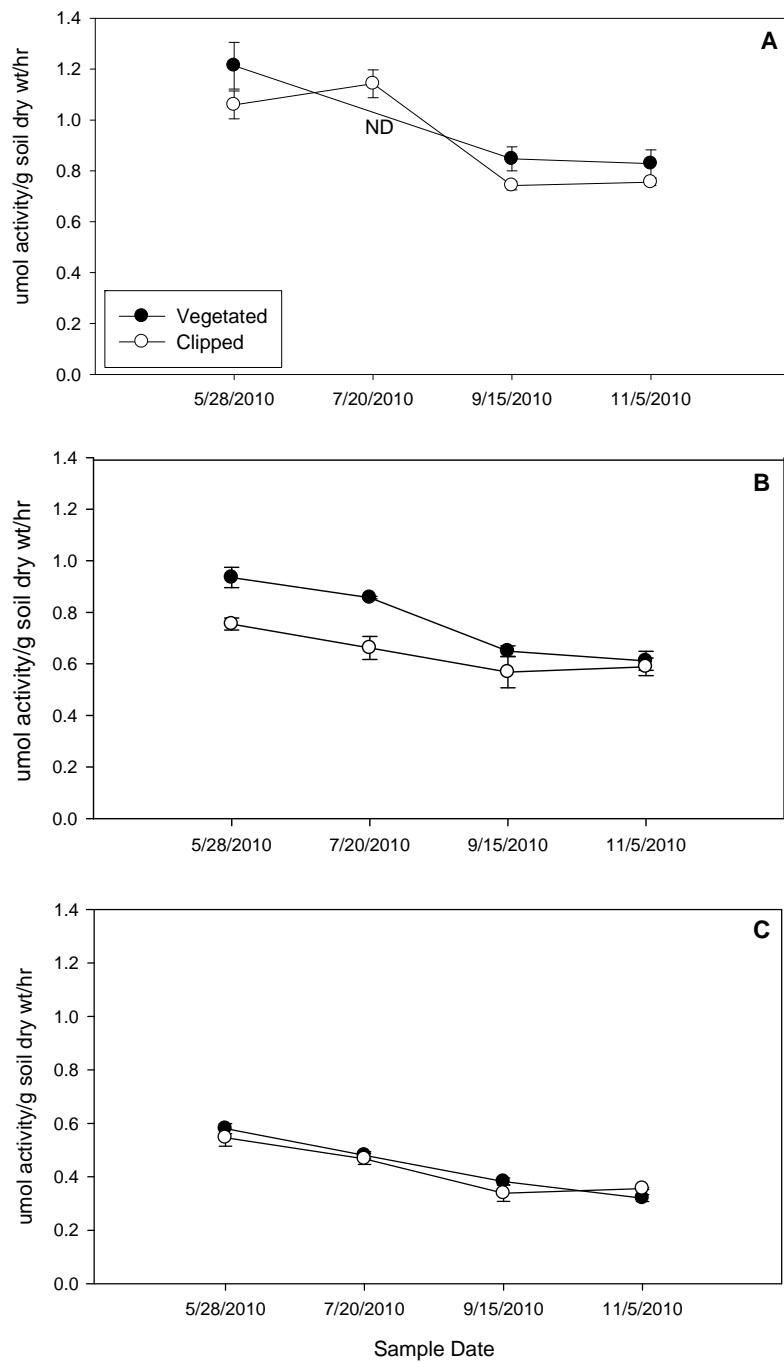


Figure 9: Mean \pm standard error for extracellular enzyme activity of β -1,4-cellobiosidase in the (A) Wet Site, (B) Intermediate Site, and (C) Dry Site for each month assessed. ND = no data available

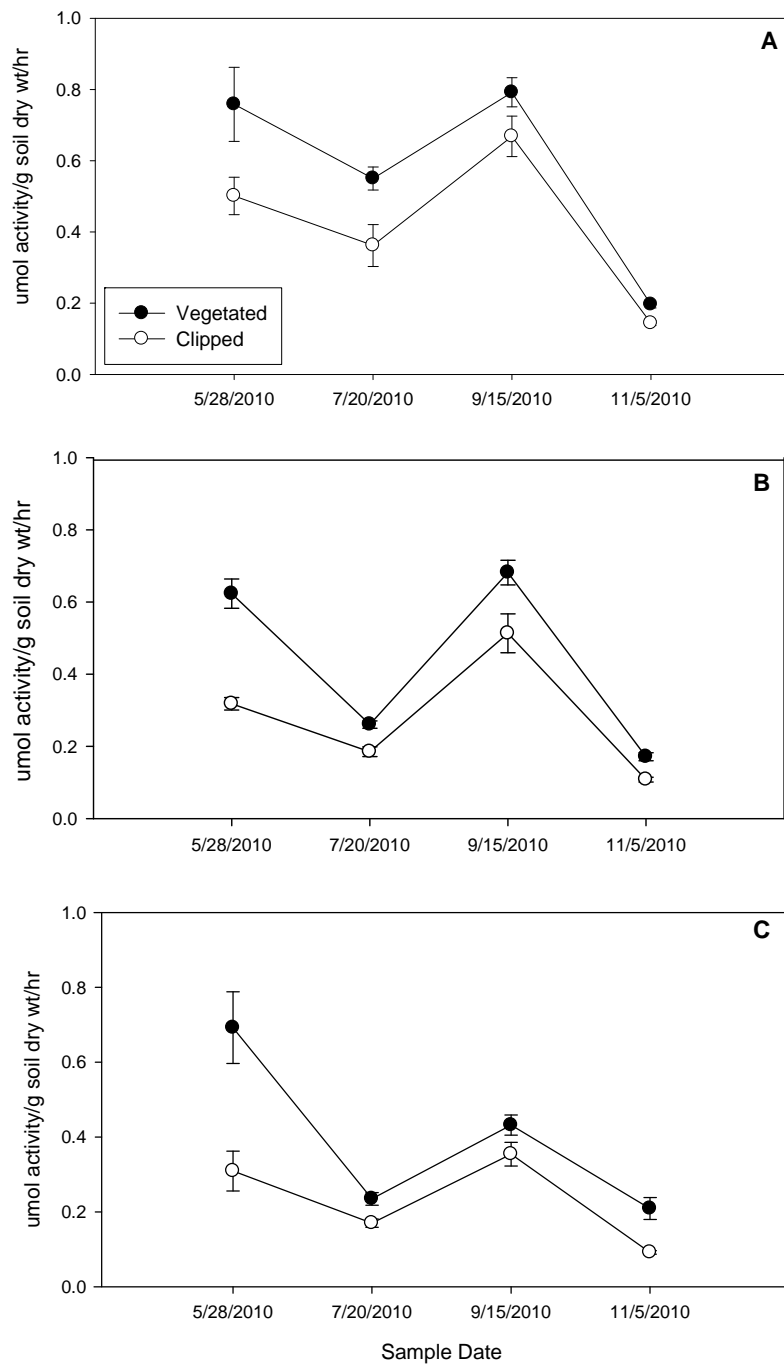


Figure 10: Mean \pm standard error for extracellular enzyme activity of β -D-xylosidase in the (A) Wet Site, (B) Intermediate Site, and (C) Dry Site plot for each month assessed.

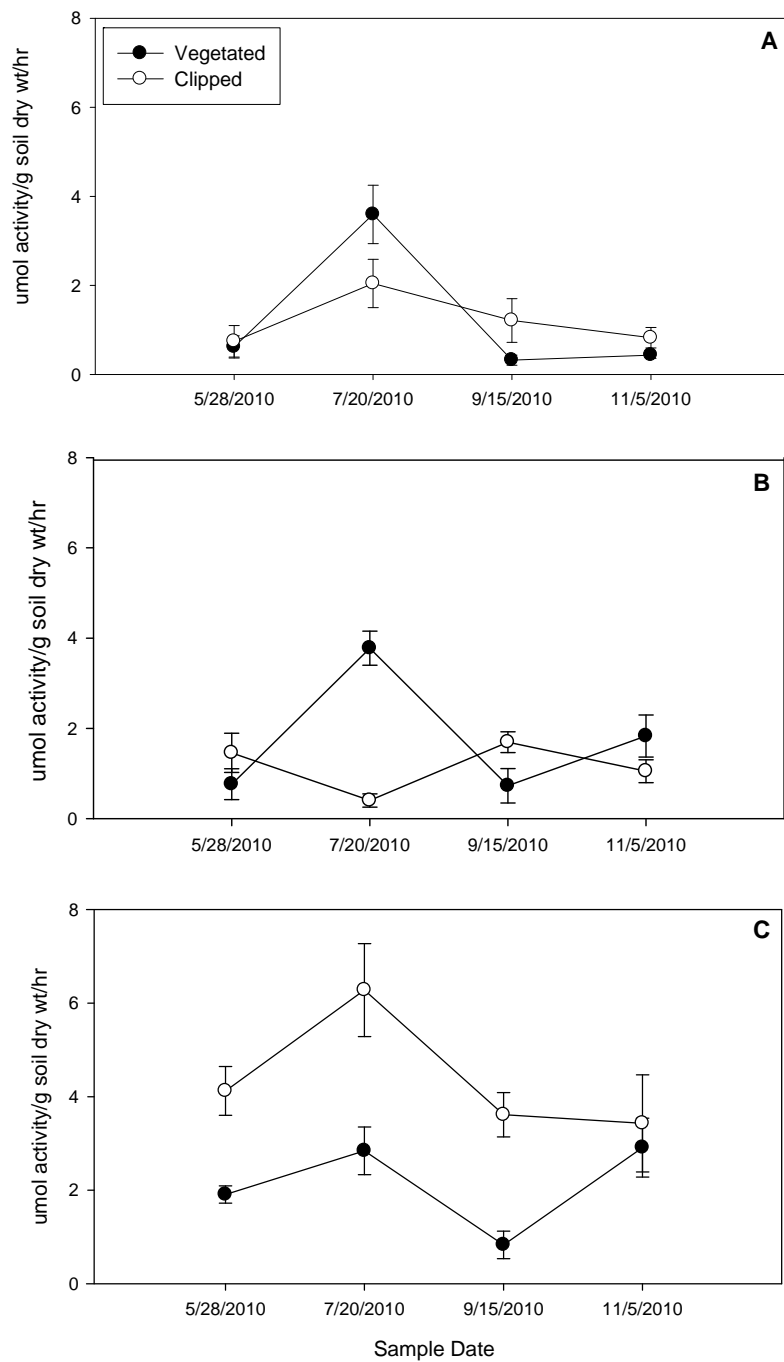


Figure 11: Mean \pm standard error for extracellular enzyme activity of Phenol Oxidase in the (A) Wet Site, (B) Intermediate Site, and (C) Dry Site for each month assessed.

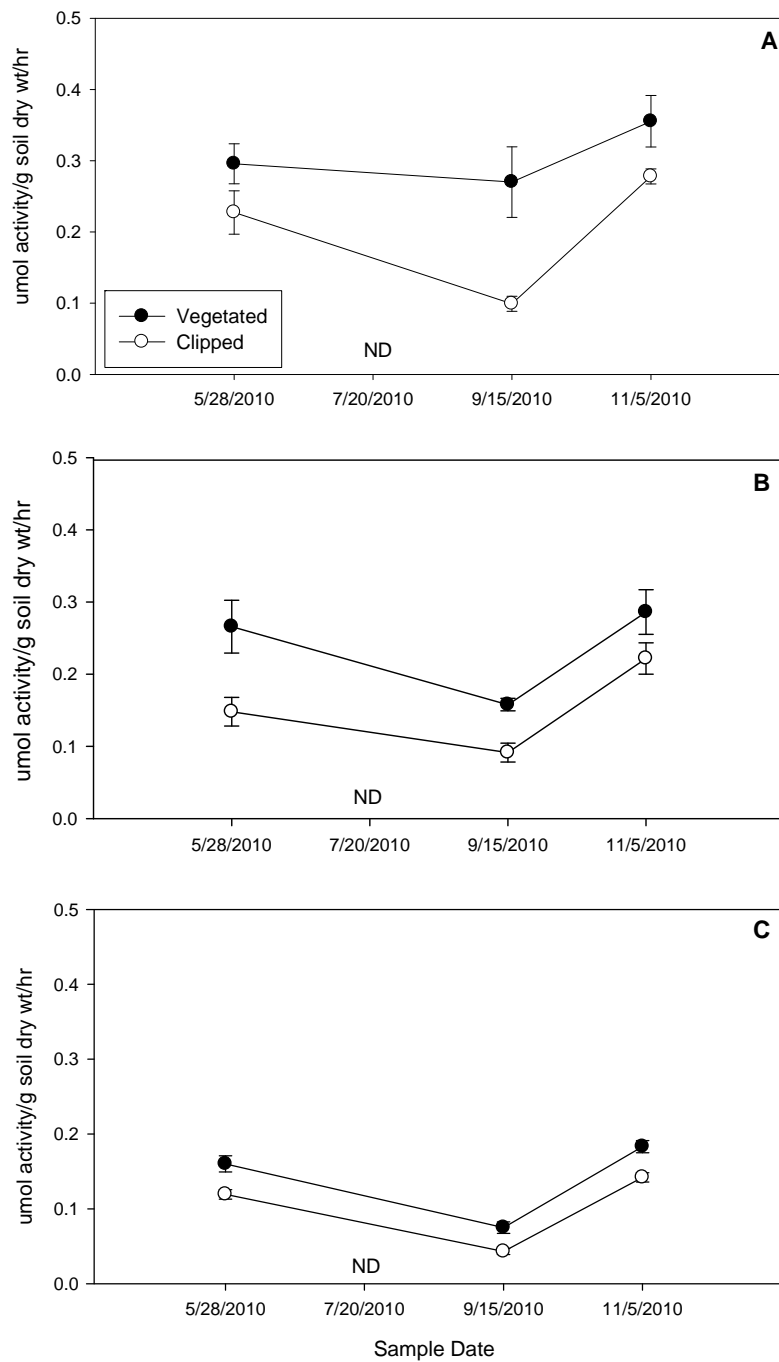


Figure 12: Mean \pm standard error for extracellular enzyme activity of Arylsulfatase in the (A) Wet Site, (B) Intermediate Site, and (C) Dry Site for each month assessed. ND = no data available

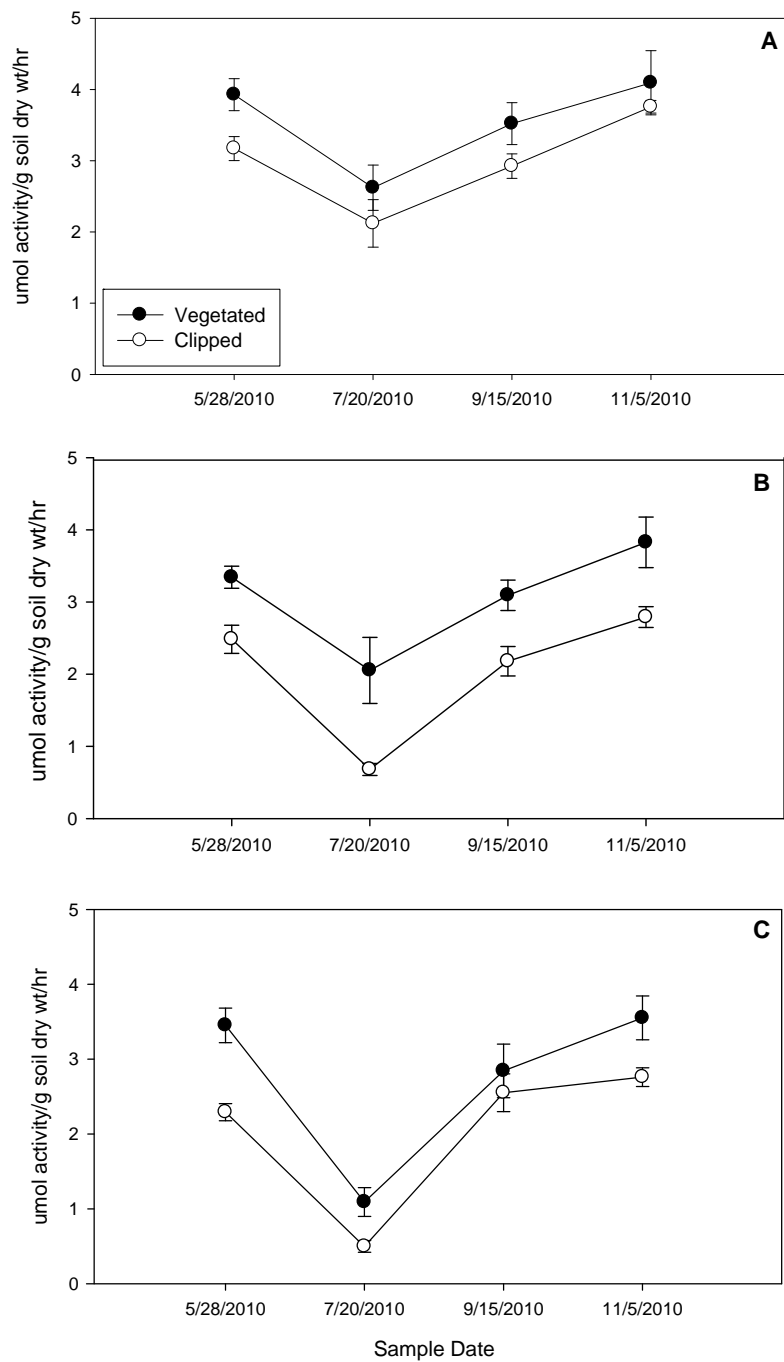


Figure 13: Mean \pm standard error for extracellular enzyme activity of alkaline phosphatase in the (A) Wet Site, (B) Intermediate Site, and (C) Dry Site for each month assessed.

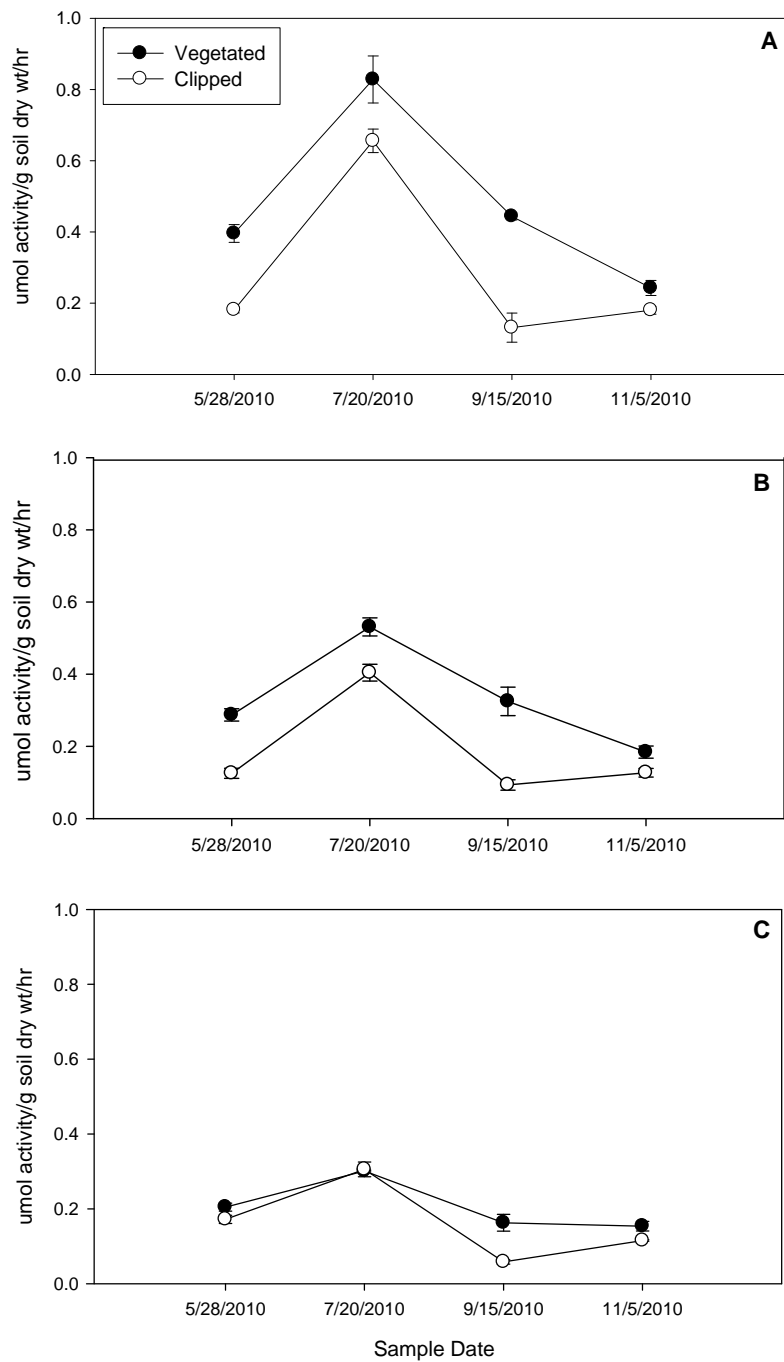
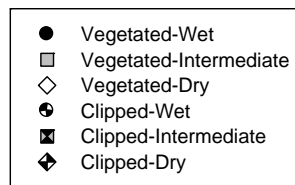
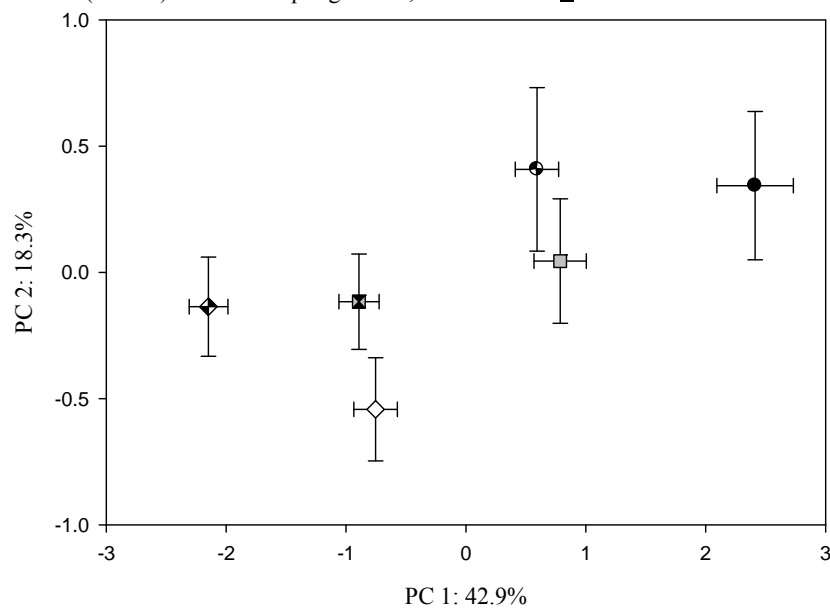


Figure 14: Mean \pm standard error for extracellular enzyme activity of leucyl aminopeptidase in the (A) Wet Site, (B) Intermediate Site, and (C) Dry Site for each month assessed.

Figure 15: PCA of all extracellular enzymes. Individual points are centroids (N = 20) with all sampling events, and error bars \pm SE



Factor Loadings

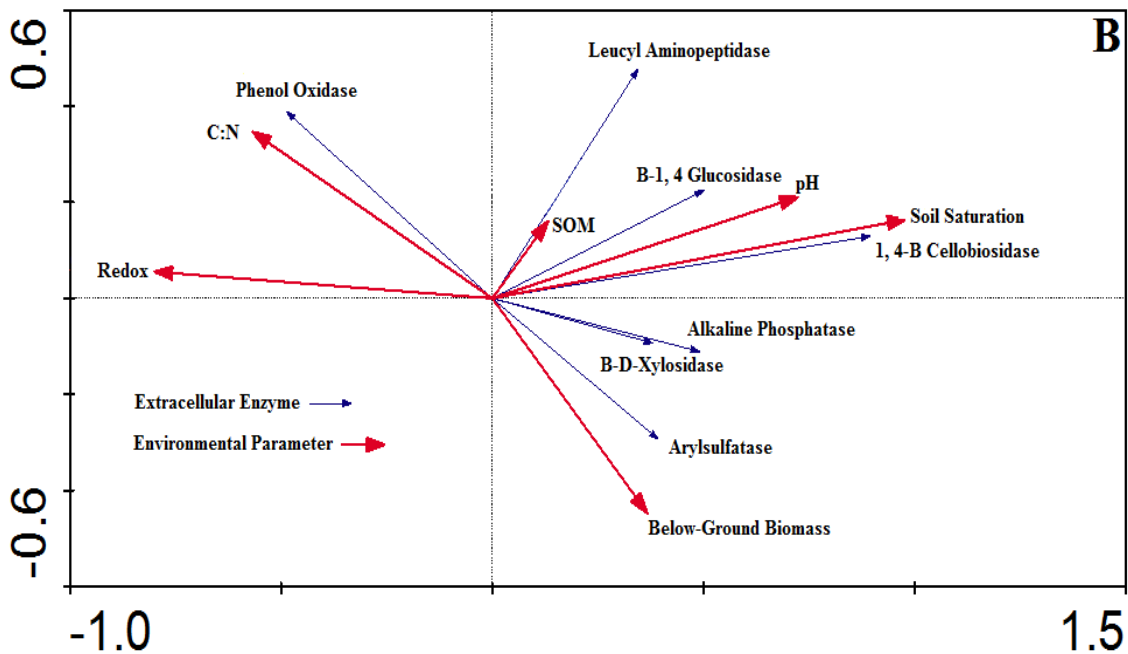
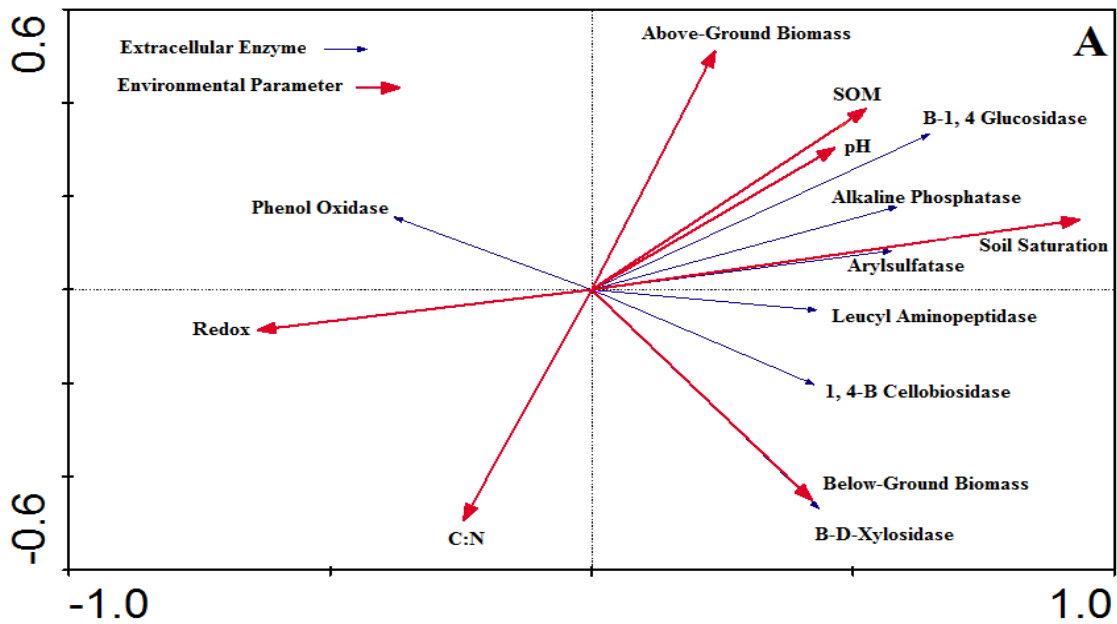
PC 1:

β -1,4 glucosidase (0.83)
 Alkaline phosphatase (0.75)
 Arylsulfatase (0.74)
 1,4- β - cellobiosidase (0.72)

PC 2:

Leucyl aminopeptidase (0.68)

Figure 16: Redundancy Analysis (RDA) correlation biplot with all extracellular enzymes as response variables (Thin Arrows) and all environmental variables (excluding temperature) as explanatory variables (Thick Arrows).
(A) -Vegetated Treatments, (B) - Clipped Treatments



Vita

Aaron James Porter was born on July 6, 1983, in Richmond, Virginia, and is an American citizen. He graduated from Patrick Henry High School, Ashland, Virginia in 2001. He received his Bachelor of Arts in English from Virginia Tech University, Blacksburg, Virginia in 2005. He then received a Master of Science in Environmental Studies from Virginia Commonwealth University, Richmond, Virginia in 2011.